



# ABSTRACT BOOK

**SETAC EUROPE 34<sup>TH</sup> ANNUAL MEETING**

5-9 MAY 2024 | SEVILLE, SPAIN

*SCIENCE-BASED SOLUTIONS IN TIMES OF CRISIS: INTEGRATING SCIENCE  
AND POLICY FOR ENVIRONMENTAL CHALLENGES.*



# Abstract Book

SETAC Europe 34<sup>th</sup> Annual Meeting

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This book compiles the abstracts from the 34th annual meeting of the Society of Environmental Toxicology and Chemistry – Europe (SETAC Europe), conducted from 5–9 May 2024 in Seville, Spain.

The abstracts are reproduced as submitted by the author and accepted by the scientific committee. They appear in order of abstract code and alphabetical order per presentation type. The poster spotlight abstracts are included in the list of poster abstracts. The presenting author of each abstract is highlighted in bold.

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Society of Environmental Toxicology and Chemistry Europe (SETAC Europe)



# About SETAC

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, managers, engineers or others interested in environmental issues. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in North America in 1979 to fill the void and quickly saw dynamic growth in the Society's membership, meeting attendance and publications.

A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The Society by-laws mandate equal representation from these three sectors for officers of the World Council and Geographic Unit Boards of Directors and Councils, and in the composition of committees and other society activities. The proportion of members from each of the three sectors has remained nearly equal over the years.

The Society is concerned about global environmental issues. Its members are committed to Environmental Quality Through Science®, timely and effective communication of research, and interactions among professionals so that enhanced knowledge and increased personal exchanges occur. Therefore, SETAC publishes two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations. Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies.

SETAC's growth is reflected in the founding of Geographic Units around the world. SETAC Europe was established in 1989 as an independent organisation, followed by SETAC Asia-Pacific in 1997 and SETAC Latin America in 1999. In 2002, the four existing organisations joined together under the governance of the SETAC World Council. SETAC Africa is the most recent Geographic Unit, which was adopted in 2012. As evidence of international acceptance of the SETAC model and of the great interest at the local level, regional chapters and branches have emerged in a number of countries.

SETAC publishes two journals, *Environmental Toxicology and Chemistry* (ET&C) and *Integrated Environmental Assessment and Management* (IEAM). ET&C is dedicated to furthering scientific knowledge and disseminating information on environmental toxicology and chemistry, including the application of these sciences to risk assessment. Integrated Environmental Assessment and Management focuses on the application of science in environmental decision-making, regulation and management, including aspects of policy and law, and the development of scientifically sound approaches to environmental problem solving. Together, these journals provide a forum for professionals in academia, business, government and other segments of society involved in the use, protection and management of the environment for the enhancement of ecological health and human welfare.

SETAC books provide timely in-depth reviews and critical appraisals on scientific subjects relevant to understanding a wide range of contemporary topics pertaining to the environment. These include any aspect of environmental chemistry, toxicology, risk assessment, risk management or environmental policy.

SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1992, and in Brussels, Belgium, established in 1993.

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## Track 1. Environmental and Human Toxicology: From Molecules to Organisms, from Omics to in Vivo

### 1.01 Advancing the Use of Effect-Based Approaches for Water Quality Assessment

#### 1.01.T-01 Use of estrogen receptor cell bioassays to evaluate dietary and aqueous exposure routes to determine the cause of widespread intersex in Largemouth Bass in a waste-water dominated stream.

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The Santa Ana River flows within the largest river basin in southern California, home to over 5 million residents. Due to upstream dams and dry climate, the surface flow is dominated by wastewater effluent most of the year, with more than 93 million gallons of effluent discharged into the river daily. The continuous input of treated effluent raises concern for protecting native fish within the river, such as the federally threatened Santa Ana sucker (*Catostomus santaanae*). Previous work in the Santa Ana River demonstrated that the estrogenic activity of water samples collected downstream of wastewater treatment plants (WWTPs) did not significantly differ compared to other sampling sites along the river. However, a 2019 survey of Largemouth bass (*Micropterus salmoides*) found that the percent of fish with the visible presence of both testes and ovaries increased with proximity to a WWTP outflow (Adjusted R-squared: 0.854, p-value: 0.015). In order to determine the contribution of dietary exposure to estrogenic substances, water, sediment, periphyton, and macroinvertebrates, were sampled and chemical and Estrogen-Receptor Bioassays were conducted on extracts. Histological analysis of Male Largemouth bass testes found that males at all sites displayed the presence of ova-testes ranging from 33-67% presence depending on the site. However, Intersex prevalence was not statistically different between sites (Chi-square test,  $p = 0.7$ ). Chemical analysis found that the estrogens 17 $\beta$ -estradiol (E2) and estrone (E1) were detected at three and four of the six river sites, respectively. Comparisons of analytical chemistry with ER-Bioassays indicated significant correlation with an r-value of 0.58 ( $p < 0.0015$ ). Evaluations of extracts of periphyton and macrofauna did not have detected estrogenic activity nor E1 or E2. These data suggest dietary exposure may not be responsible for the widespread intersex observed in fish of the SAR.

#### 1.01.T-02 Chemical mixtures : Additivity and beyond

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Semi-quantitative and untargeted screening methods mean it is now more tractable to detect and measure a wider range of chemicals present in the environment. The Environment Agency use semi-quantitative GC-MS and LC-MS methods to measure >1,000 chemicals in groundwater and surface water samples collected from across England and Wales. These measurements indicate that in any given sample, up to 100 chemicals may be detected. The risks to aquatic ecosystems from these mixtures can be assessed by comparing measured concentrations to hazard values derived from toxicity tests. Such analyses indicate that, as expected, risk increases with the number of detectable compounds. However in the vast majority of cases, much of the risk (>80%) is due to  $\leq 5$  of the chemicals present. This analysis assumes, as a default, that the chemicals work additively. What if this is not the case? Experimental studies have shown cases of both antagonism and synergism in the joint toxicity of chemical pairs. A meta-analysis indicated that such non-additive cases represent about 20% of all mixture studies. Interactions occur at even higher frequency between pesticides, with azole fungicides often one of the two chemicals in these non-additive mixtures. The capacity of these fungicides to inhibit cytochrome P450 enzymes, likely contributes to these cases of synergism, with species with a low CYP P450 diversity particularly affected by such interactions (i.e. such species show more frequent and larger magnitude synergism when exposed to a chemical mixture that include an azole). If this synergism potential is included in the risk assessment for surface water for chemical mixture effects, then the number of locations at risk can be greatly increased.

#### 1.01.T-03 Can we use a biosensor to detect water pollution in real-time? Results of a pilot study in the Venice lagoon

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In 2021, 214 million tons of chemicals hazardous to health were produced (4% more than in 2020; 1% more than in 2019), along with 85 million tons of chemicals hazardous to the environment (6% more compared with 2020; 2% more than in 2019) (EUROSTAT). A fraction of these chemicals reaches surface, ground, and marine waters in complex mixtures through different emission sources: industry, WWTPs, diffuse sources (leaching and runoff in agricultural fields, air deposition, etc.). In this challenging context, the European Commission adopted a proposal to review and modify the Water Framework Directive in 2022, in line with the objectives of the European Green Deal and the Zero Pollution Action Plan. To address these new stringent water quality requirements, we have developed a new monitoring device, called WATERSCAN that continuously tests water quality using a biosensor. WATERSCAN applies real-time PAM (Pulse Amplitude Modulated) fluorometry to detect changes in functional indicators of microbial biofilms exposed to a constant water flow and includes a

local clean reference. The device also included passive samplers POCIS (Polar Organic Chemical Integrative Samplers), specific for polar organic compounds, Diffusive Gradient in Thin film (DGT), for trace metals and a novel passive sampler to determine chemotaxonomical phytoplankton composition. The prototype, protected by a patent, has been tested in the field. Here are the results of the pilot study conducted at the Arsenale of the Venice lagoon from 17/7/23 to 27/7/23. First results indicate that the device can operate with minimal maintenance and provides highly sensitive results with a constant water flow during several days. Significant differences were detected between monitoring and reference chambers indicating possible water contamination events, in line with the results of passive sampling. A local reference was essential to discriminate significant divergences in PAM signals, especially during and after a storm event. The analysis of chlorophyll-a content indicates higher concentrations in the inflow compared to the outflow. This pattern is more pronounced during the afternoon, aligning with the day/night cycle of phytoplankton. HPLC and microscopy analysis confirm that colonization of the biofilm by external species is reduced to a minimal colonization by diatoms. Future developments will include the implementation of a standard monospecific biofilm to reduce variability in results.

#### **1.01.T-04 Increasing the Acceptance of Effect-Based Methods for Surface Water Quality Assessment – Results of an Interdisciplinary Workshop**

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Several Effect-Based Methods (EBM) are currently available to characterise water pollution. However, they are only partially represented in the relevant regulations for assessing water quality in Europe. In many of them, EBM are not accepted, often not even mentioned. The main reason identified was the lack of regulatory acceptance. Chemical analysis is often seen as simpler, more accurate and safer, and also as a competitor rather than an explanatory tool to explain the observed effects. Lack of knowledge about EBM, including its advantages such as higher sensitivity or integration of mixture effects, is another potential reason. Further reasons are doubts about the robustness of the data and their (ecological) relevance. Lack of standards for the individual assays and of experience of the regulatory laboratories, are potential reasons for the missing acceptance and establishment in water management practice or regulation. To overcome this problem the participants of a workshop, organised by the Association of German Engineers (VDI) and the North Rhine-Westphalia Office of Nature, Environment and Consumer Protection (LANUV) suggested several measures: (i) Intensive communication is needed not only between the scientific community and the authorities involved, but also between the EU and the respective national administrations, (ii) implementation of EBM in the respective guidance documents, (iii) the advantages, but also the differences, of EBM compared to standard analytical methods should be better explained to government and policy makers. (iv) interlaboratory comparisons are needed to standardise technical procedures such as sample preparation and extraction as well as EBM, (v) for the results of EBM to be admissible in court, the derivation of toxicity thresholds/ effect-based trigger values must be scientifically robust and accepted by regulatory authorities, (vi) the assays and the organisms used in these assays need to be available in sufficient quantity and quality. In addition, (vii) EBM-related data should not only be published in peer-reviewed scientific journals, but also in journals relevant to government, water management professionals and the private sector. Also (viii) competence centres for co-ordinating existing initiatives should be established. As a first step towards an EU-wide application, the current proposal to amend the Water Framework Directive proposes to include EBM to assess the presence of estrogenic hormones in water bodies.

#### **1.01.T-05 Daphnia magna behavioural responses. An emerging novel tool for effect direct analyses of neuro-active substances in waste water treated effluents**

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Behavioral responses in non-animal species, such as *Daphnia magna* and zebrafish embryos, are increasingly used as sublethal toxicological endpoints for assessing neurodevelopmental and neuro-disruptive effects of toxic contaminants. However, their reliability in detecting neuro-active compounds in real-world field situations remains unclear. In the present study, we present the we present the outcomes of a battery of seven behavioral responses in *D. magna*, along with neurotransmitters and related metabolites, employed to identify neuro-active contaminants present in wastewater-treated effluent discharges from three rivers in southeastern (SE) Spain. The investigated rivers include Llobregat and Besós, located within the Barcelona urban area and receiving a mix of industrial and domestic effluents, and the Onyar river in Girona, primarily affected by agricultural activities. Our sampling involved collecting water samples from wastewater treatment plant (WWTP) effluent discharges, upstream and downstream locations, on two occasions. Contaminants from these samples were extracted and pre-concentrated using solid phase extraction (SPE) cartridges, and tested at concentrations enriched by factors of x2, 5, 10 and x20 fold. Analysis of contaminants was performed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Of the 48 water samples analyzed at increased concentrations, 43 showed impacts on at least one of the measured responses. Specifically, Llobregat, Besos and Onyar rivers, respectively, affected 24, 19 and 2 *D. magna* responses. The extracts concentrated x20 fold accounted for 67% of the detected effects, clearly indicating a concentration-dependent response. Additionally, WWTP discharges had a pronounced impact on the studied rivers, contributing to 88% of the observed

effects. Finally, the use of chemometric and bioinformatic tools helped to identify potential neuro-active contaminants and understanding their modes of action.

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### 1.01.P Advancing the Use of Effect-Based Approaches for Water Quality Assessment

#### 1.01.P-We001 Application of a bioassay battery to assess water quality in 15 Swiss watercourses

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Effect-based methods such as ecotoxicological bioassays and biomarkers are promising for assessing water quality. As screening tools and early indicators, they provide an important bridge between exposure (i.e. the chemicals present and the associated risks to aquatic organisms) and the effects on organisms in the environment and enable the assessment of mixtures of contaminants in environmental samples, especially because not all substances present can be measured. The Ecotox Centre has carried out an extensive monitoring study with the aim of extending the assessment of water quality using a selection of effect-based methods. To this end, water quality was assessed at sites with extensive, agricultural or agricultural-urban land use using a comprehensive bioassay battery of largely standardised water and sediment bioassays. Molecular biomarkers in young brown trout were used in streams in extensively and agriculturally used areas. Together with measurements of abiotic parameters and chemical analyses, a comprehensive picture of the influences can be obtained. The current presentation provides an overview on the results of the aquatic bioassays.

Fourteen largely standardised *in vitro* and *in vivo* bioassays were used with different cell lines, algae, aquatic invertebrates (ostracods and water fleas) and fish embryos and larvae. The risk to aquatic life was assessed using effect-based, bioassay-specific thresholds. Potentially problematic sites were identified. Results of this effect-based risk assessment were also compared with the mixture risk assessment based on chemical measurements. Sites with agricultural-urban land use showed the strongest effects, followed by sites with agricultural land use and sites with extensive land use. Bioassays that showed the most exceedances of effect-based thresholds were reporter gene tests for pollutant perception and oxidative stress as well as the combined algae test (endpoint growth) and acute toxicity tests using fish embryos and a fish cell line. The comparison with established assessment methods based on chemical analytical data has shown that these effect-based methods complement each other and the chemical analysis and thus improve the detection of ecotoxicological risks. Results presented here will be used to propose suitable test batteries adapted to the sources of pollution in Switzerland.

#### 1.01.P-We002 How land-use influences aquatic ecotoxicity

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A major stressor complex to aquatic ecosystems is the steady increase of micropollutants over the last decades.

Micropollutants are particularly problematic for the environment because they can cause toxic effects even at very low concentrations. They have the potential to affect both the environment and human health as they are often ubiquitous and persistent. Micropollutants are anthropogenic organic trace substances and comprise, for example, pharmaceuticals, pesticides, biocides and a wide range of household and personal care products and therefore have multiple pathways into the environment. In this study field samples were analysed using different *in vivo* and *in vitro* effect-based methods (EBM). The overall aim was to attribute the measured effects of the different EBMs to specific pollutant sources. This knowledge should allow a more targeted prior test selection for the evaluation of field samples.

30 water and sediment samples from three different streams in the heavily anthropogenically impacted southern Rhine-Main area were analysed. All samples were tested *in vitro* for their endocrine and dioxin-like activity, mutagenicity, baseline toxicity and oxidative stress. For *in vivo* assessment the snail *Potamopyrgus antipodarum*, the crustacean *Gammarus fossarum* and different macrophytes were tested in chronic exposure. For all sampling sites the surrounding land use (arable land, forest, pasture, urban area) was recorded based on the Corine Land Cover 5 dataset and upstream discharger of waste water treatment plants (WWTP) were identified. To evaluate the samples, the ecotoxicological effects were first compared with the main land use type. A PCA was then used to identify in more detail the main influencing factors at each sampling site and again compared with the results of the EBM. Overall, the selected test systems were well suited for an effect-based quality assessment of the rivers in general and thus for a first assessment of the hazard potential of existing pollutants. It was difficult to link the effects of a given EBM to one particular type of land use, as the causes of water pollution appear to be multi-causal. Agricultural pollutants may have been underestimated, as they are often not captured by grab samples. However, there were clear correlations between the effects of EBM and the proportion of the pollution load from upstream WWTP.

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### **1.01.P-We003 Evaluation of disinfection by-products (DBPs) and related water sources using a panel of effect-based bioassays**

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Water systems worldwide are confronted with a complex mixture of thousands of known and unknown (unregulated) emerging compounds. Furthermore, water systems and treatment technologies face a major challenge and are under great pressure to deliver safe and affordable water services to a growing population. The SafeCREW project catalyzes innovations in several European water treatment sites through improved combinations of natural and engineered treatment systems. Water quality and treatment performance is generally assessed for a limited set of individual parameters, possibly resulting in an incomplete quality assessment. Room is now given in the Drinking Water Directive to develop a risk-based monitoring program. In the SafeCREW project we applied a comprehensive panel of human cell-based biological detection methods (i.e. CALUX<sup>®</sup> assay) to assess the impact of disinfection by-products (DBPs), related chemicals and chemical mixtures on a range of key types of toxicity pathways (e.g. cytotoxicity, genotoxicity, oxidative stress, endocrine effects, PAH and PFAS--like properties and obesity) in different model and real demonstration site waters. Such effect-based bioanalysis allows for detection of known/unregulated/unknown chemicals and to evaluate a safer risk assessment of total toxicity of water samples, including mixture effects. Most relevant DBPs were selected and tested by the CALUX panel to obtain relative potency factors (RPFs). For this first round of analyses, model and real water samples of natural organic matter (NOM) proxies for ground and surface waters were selected to evaluate the response of the innovative biotests on different disinfection methods. Additional water treatment using innovative treatment technologies (oxidation, advanced oxidation) improves water quality significantly as determined using panel of effect-based CALUX bioassays. Results from effect-based CALUX bioassays can be used for evaluation of efficiency of treatment technologies to remove bioactive substances. Our here applied effect-based trigger values (EBTs) for the assessment of water quality and implementation of effect-based bioassays in regulatory water frameworks for risk assessment is discussed. The here presented action plan for water treatment plant operators based on EBT, enhances the applicability of effect-based bioassay for assessment of water quality and regulatory acceptance.

### **1.01.P-We004 COMPLEMENTARY CHEMICAL SCREENING + BIOASSAY APPROACH FOR MONITORING WATER QUALITY: PRESENTATION OF A CASE STUDY**

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Today, the microbiological and chemical quality of drinking water is a major concern, particularly contaminants of emerging concern (CECs). The occurrence of these emerging pollutants in the environment has been a proven fact for several years now, but their impacts and risks, at the concentrations usually found in environmental matrices, are still poorly estimated.

The strategy put in place by VEOLIA Research and Innovation consists of carrying out parallel chemical and bioassays to monitor of various drinking water treatment processes as well as the resource:

- On one site, the implementation of two "semi-targeted" complementary chemical analysis protocols (SBSE-GC-HRMS and SPE-LC-HRMS) to the water samples. Next, a major data reprocessing operation is carried out, using in-house tools, to identify the substances present among almost 4,000 micropollutants.
- and other site, the implementation of analyses based on the biological activity or toxicity induced by any micropollutants present in the extract of the sample analyzed (effect-based monitoring, EBM). The 4 most relevant modes of action to monitor in drinking water supply according to the experts (GWRC) are: endocrine disruption, oxidative stress, xenobiotic metabolism and genotoxicity.

This paper presents the results of a case study. This combined Chemical Screening + Bioassay approach has been used to monitor the performance and evolution over time of various samples from the New Goreangab Reclamation Plant (Direct Potable Reuse Plant) in Windhoek, Namibia.

The results of chemical screening highlight that most of the micropollutants identified in raw water are Pharmaceuticals and Industrial contaminants and the removal performance of the whole treatment line is very good. The results of Bioassays highlight the decrease in toxicity during treatment, different levels of toxicity between the two campaigns and the presence of very low toxicity in the water produced (Estrogenic effect).

Water (even in the resource) contains micropollutants, at a high or low level of concentration. For the moment, only targeted chemical analysis of certain micropollutants is regulated, Bioassays are not. This study shows how Chemical Screening and Effect-Based Monitoring are complementary for a better water quality risk assessment. Chemical analysis is still essential to understand which micropollutants are present in the samples, and the main advantage of EBM is to study their potential mixture effects on human health.

### **1.01.P-We005 Implementing effects-based monitoring strategy to assess water quality in California waterbodies**

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There is a growing interest in the application of cell-based bioassays to supplement chemical-by-chemical monitoring and assess the biological impacts of complex environmental mixtures. In recent years, California agencies have invested in the

development and field evaluation of cell bioassays (e.g., estrogen receptor, aryl hydrocarbon receptor and glucocorticoid receptor assays) for water and sediment quality screening. This poster presentation will highlight the three-step approach we have employed to help facilitate the incorporation of cell bioassays in ambient monitoring programs in California. The first step focused on the development of performance-based criteria and data quality plan to ensure that all data reported meets minimum requirements. The second step focused on large scale application of cell bioassays and comparisons with other lines of evidence. This step was critical in demonstrating the added value of cell bioassay data. The third step focused on the development of a tiered interpretation framework with several cell bioassay thresholds (or effects-based triggers) associated with potential management actions.

#### **1.01.P-We006 Effect-based assessment: Seasonal differences in streams**

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The majority of aquatic ecosystems are now massively degraded as a consequence of various anthropogenic impacts, including pollution. Chemical analyses are often used to identify key stressors, but these selective testings represent only a fraction of the substances actually present in water bodies. However, for effective water management, the relevant pollutants need to be clearly identified. Effect-based methods (EBM) allow an integrative assessment of the pollutant effects of even complex mixtures as they occur in the environment, including potential interactions between substances. Therefore, they have the ability to bridge the gap between ecological condition and chemical pollution, thus providing a better understanding of the degraded status of water bodies. In this study different sites were sampled four times during the year and analysed via *in vitro* EBMs. The overall aim was to identify seasonal differences of the ecotoxicological activity in water samples. This knowledge should allow a more standardized evaluation of field samples.

A total of 15 water samples were collected in the southern Rhine-Main area. Due to various inputs and structural deficits, the water bodies there do not achieve a good ecological status as defined by the European Water Framework Directive (EU-WFD). Each site was sampled four times in 2021 (spring, summer, autumn, winter) and tested *in vitro* for endocrine and dioxin-like activity, mutagenicity and baseline toxicity. The effects found were first tested for differences in the time of sampling. Across all sites, no seasonal differences were found for the majority of the tests performed. However, the analysis of individual sampling sites showed site-specific seasonal variations in ecotoxicological effects. Therefore, different complex exposure scenarios are to be expected spatially and temporally, so that future ecotoxicological water assessments should consider not only factors such as a standardised sampling method, but also comparable sampling periods. For further evaluation, the effects were converted into a five-level system from "very good" to "poor" in order to determine an ecotoxicological water quality class for each sampling site. This was used to identify the causes of deficient water conditions, thereby helping to identify the reasons for the failure to achieve good ecological status.

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#### **1.01.P-We007 How Much Do Commonly Monitored Organic Contaminants Explain Species-Specific In Vitro Toxicity of Seawater?**

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The Indo-Pacific finless porpoise and Indo-Pacific humpback dolphin are iconic threatened marine mammals in Hong Kong. Chemical pollution is amongst the multiple stressors that impact their health. While there have been studies successively devoted to different groups of contaminants in the seawater where the porpoise and dolphin inhabit, it remains elusive to what extent these commonly monitored contaminants contribute to the overall toxicity of seawater and whether there is an imperative to resolve other contributing toxic pollutants. To fill this knowledge gap, we developed species-specific cell lines to assess the cytotoxicity of Hong Kong's marine water and employed mixture-toxicity modeling to determine the quantitative role of 32 chemicals previously detected thereof, including polycyclic aromatic hydrocarbons, polyfluoroalkyl substances, brominated flame retardants, organotins, ultraviolet filters, and algal toxins. These chemicals collectively accounted for a greater proportion of seawater cytotoxicity in the northern, eastern, central, and southern waters (25-43%) than in the western waters (6%). Consistent to this pattern, pectenotoxin-2, which is produced by *Dinophysis* spp. and the major driver of all the studied chemicals, explained 22-36% of seawater cytotoxicity in most of the waters except for the western waters (4%). In contrast, di-butyltin, one component of the antifouling agents in shipping activities, contributed consistently to seawater toxicity across the coastal waters (2-6%). These results pinpointed zone-specific focus in identification of marine pollutants potentially impacting the cetacean health (e.g., more on anthropogenic chemicals in the western waters vs. more on natural biotoxins in the rest of the coastal waters) by suspect/non-target screening combined with artificial intelligence-facilitated toxicity profiling. Similar approaches should be directed towards chemical mixtures accumulated in the porpoise and dolphin. The development of effect-based trigger values for mixture effects should be prioritized as a tool for managing the overall risk of chemical cocktails in coastal waters to protect these threatened marine mammals.

### **1.01.P-We008 "Chemical characterization and genotoxic assesment of treated and untreated municipal wastewater from WWTPs of Las Palmas de Gran Canaria (Spain) and Mahdia (Tunisie) cities"**

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The quality monitoring of municipal wastewater has an interesting role in sustainable development. In fact, wastewater treatment plants (WWTPs) constitute the main source of emerging pollutants which are released into the environment depending on their resistance to conventional treatments. These emerging and recalcitrant pollutants present a certainly environmental impact and risks to human health. A recent approach in environmental protection involves the toxicological assessment of effluents to complement the usual chemical evaluations. In our present work we effected a comparative study between two WWTPs of Las Palmas de Gran Canaria (Spain) and Mahdia (Tunisie). Results indicated that the physicochemical analyzes carried out for parameters like COD, BOD, TSS, TOC, DOC, POC, MO, conductivity, turbidity, etc. showed that both the influent and the effluent of the WWTP of the Las Palmas de Gran Canaria seem to be more contaminated than those corresponding to WWTP in Mahdia which show lower values of the same parameters. However the genotoxic investigation carried out by the SOS chromotest with *E. coli* PQ37 in the presence and in the absence of the metabolic activation system (S-9) shows that the influent and effluent of wastewater treatment plants of Mahdia induced strongly genotoxic effect compared to those of Las Palmas de Gran Canaria. In fact, the IF is dose dependent and reaches a value of 5.32 with the highest concentration (well above the genotoxicity barrier of 1.5) in influent of the WWTP of Mahdia. This genotoxic effect decrease but remains greater than 1.5 at the effluent of the WWTP. We can conclude that the physicochemical parameters do not reflect the toxicity in the case of the effluent collected from WWTP in Las Palmas de Gran Canaria and that the genotoxic power of the influent and effluent of WWTP samples of Mahdia could be attributed to the presence of higher quantities of organic compounds cocktails which resist to the lagoon treatment system used. The genotoxic power of the tested wastewater treatment plants samples could be attributed partly to the presence of higher quantities of antibiotics which were detected in these samples and included in another of our works.

### **1.01.P-We009 Assessing Estrogenic Activity in Complex Environmental Matrices: The Impact of Organic Matter and Suspended Particulate Material on the Yeast Estrogen Screen**

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The presence of estrogenic endocrine disruptors in aquatic environments has been a concern and bioassays are recommended tools for their monitoring. However, the physicochemical properties of contaminants and the environmental matrix features may influence the observed response. The present study aimed to assess this influence on the Yeast Estrogen Screen (YES) assay. Mixtures of 17 $\beta$ -estradiol (E2) and humic acid (HA) were evaluated through the Schild approach aiming to investigate the interactions between estrogens and dissolved organic matter (DOM). Moreover, environmental samples from municipal landfill leachate and wastewater treatment plant (WWTP) influents and effluents were screened for estrogenic and antiestrogenic activity at both dissolved and particulate (SPM) phases. Physicochemical characterization for solids and organic matter was also performed and a principal component analysis (PCA) was run, aiming to investigate global relationships between results and to probe if the assessed parameters were indicative of an estrogenic activity distribution. The HA test concentrations had strong apparent antagonistic effect and reduced the E2 response, even at low levels. Humic substances may not only reduce estrogen bioavailability, but also interfere with the assay mechanism through enzymatic inhibition thus masking the sample estrogenic potential. DOM-rich environmental samples may have their estrogenic potential underestimated by the YES assay and analogous methods. Landfill leachate had total E2-Equivalents (E2-Eq) in the range 1282–2591 ng L<sup>-1</sup>, while WWTP samples were in the range 12.1–41.4 ng L<sup>-1</sup> (influent) and up to 2.3 ng L<sup>-1</sup> (effluent), respectively, so estrogenicity was reduced 92% in average by the treatment process. SPM phase was responsible for 33–100% of measured E2-Eq, though cytotoxicity was recurrently associated with particles >0.7  $\mu$ m. Antiestrogenic activity was observed in both phases and might also have masked the estrogenicity of samples. Physicochemical parameters supported the interpretation of E2-Eq multiphase distribution, but no pattern was confirmed by the PCA and parameters were not determinant. In conclusion, the *in vitro* YES assay is subjected to factors intrinsic to the environmental sample that can influence on the measured estrogenic response. Therefore, results interpretation should be performed together with organic matter characterization parameters, cytotoxicity and antiestrogenic activity evaluation.

### **1.01.P-We010 Water fleas as a "canary in the coal mine" to early predict water pollution**

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The growing human population has a significant impact on the environment and the planet. Therefore, assessment of environmental exposure to toxic chemicals and their impact on biological systems is important. Currently traditional approaches focus on the detection of chemicals or monitoring the abundance of fauna and flora. However, these measurements are weak in terms of providing minimal detection of a fracture of possible contaminants in the environment, while they also fail to produce a diagnostic insight concerning the type of stressor. Furthermore, these methods cannot predict any future impact before ecological damage has occurred and this is reflected in the poor legislation in some cases of pollutants, which

comes as a natural consequence of these approaches not being able to safeguard the environment. Moving away from traditional methods to monitor pollution, modern eco-toxicology is shifting towards the use of effect-based methods as key approaches to supplement and provide meaningful mechanistic insight for pollution assessment. Focusing on the freshwater ecosystem, daphnids, commonly known as water fleas, have acquired a central position in molecular ecology and ecotoxicology. Exposing daphnids in a series of pollutants and generating molecular fingerprints from their responses allows a more in depth understanding for the action of pollutants in non-target species. Expanding from acute to chronic and transgenerational exposures we are able to understand more on the impact of pollutants. Combining phenotypic endpoints with holistic metabolomic approaches we defined the molecular changes of several chemicals and novel materials in the physiology of daphnids for individual and mixtures of stressors. Comparing these “signatures” with the ones obtained from actual water samples, we transformed daphnids as an equivalent to “a canary in the coal mine” to early predict pollution and provide new metrics for water quality.

#### **1.01.P-We012 Biological activities of antidepressants and G protein-coupled receptors (GPCRs)-acting pharmaceuticals to monoamine transporters of human, fish, and water flea**

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Over recent years, growing numbers of human pharmaceuticals have been detected in effluents of wastewater treatment plants (WWTPs) and river water. Antidepressants are one of most commonly detected human pharmaceuticals, and concern about their potential risks to aquatic species has been raised because they modulate neurotransmission in nervous system. Their target molecules (i.e., monoamine transporters) are evolutionally well conserved from invertebrate to human.

So far, we demonstrated that antidepressants could inhibit not only human but also zebrafish monoamine transporters<sup>1,2</sup>. Several studies reported that antidepressants induce abnormal behaviors and alter the reproductions of invertebrates in vivo exposure test under the laboratory experimental setup (e.g., *Echinogammarus marinus*, snails, and clams). However, nobody has investigated the activity of antidepressants on the monoamine transporters of invertebrates.

In this study, we cloned SERT of water flea (*D. pulex*, WTN6) for the first time. We investigated by the in vitro assay whether SERT of water flea could be inhibited by antidepressants. We also cloned monoamine transporters of medaka and ayu fish (*Plecoglossus altivelis*), which is one of the important food fish in Japan.

As a result, antidepressants could inhibit not only human but also fish and water flea SERT. Interestingly, fish SERTs were strongly inhibited by antidepressant compared to human SERT. We also we applied the in vitro antidepressant assay to WWTP effluent extracts. All wastewater extracts showed reduction of APP uptake for not only human but also fish and water flea SERT. These results indicate that at least molecular level, fish and water flea could be more seriously affected by antidepressants in water environment than human.

#### **1.01.P-We013 Application of gene expression biomarkers in brown trout, *Salmo trutta*, to assess water quality in 10 Swiss watercourses**

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Analysis of gene expression biomarkers in resident organisms represents a promising effect-based approach for water quality monitoring. It allows a sensitive and integrated detection of environmental pressures, including contamination with micropollutants. In this study, we used microfluidic qPCR for high-throughput analysis of 96 biomarker genes in brown trout. These genes were selected to detect the effects of micropollutants, in particular pesticides, and other stressors by reflecting a wide range of biological processes. The selection was based on scientific literature and databases (e.g. AOP-Wiki, Comparative Toxicogenomics Database, EcoToxChip) as well as previously established biomarkers (Ecotox Centre in collaboration with aQuaTox-Solutions GmbH). Biomarkers were measured in brain and liver tissue of juvenile brown trout from ten sites in Switzerland, including five sites with extensive land use and five sites with agricultural land use. Depending on the site, trout parr (less than one year old) and/or one year old trout were studied. In a parallel project carried out at the Ecotox Centre, water from the same sites was analysed by detailed chemical analysis and a battery of bioassays. The biomarker results show an influence of size and sex on the expression of specific genes in the liver and brain and the need to take these confounding factors into account when interpreting the responses. Accounting for these factors, we detected biological effects (e.g. oxidative stress, effects on the immune system, endocrine disruption, effects on metabolism) at several sites, with the majority of effects attributed to sites with agricultural land use. While the chemical analysis-based risk assessment indicated a risk to vertebrates at only one of the sites, which was concordant with the biomarker results, we observed biological effects at several other sites that warrant further investigation. Our study demonstrates the potential of biomarkers to provide complementary information to chemical analysis and bioassays for the identification of problematic sites, biological effects and stressors. To fully leverage the potential of biomarkers, future research and monitoring studies should aim to establish reference values that allow to distinguish between responses indicative of a stressed or healthy state. This study represents a step forward in the use of biomarkers for biomonitoring.

#### **1.01.P-We014 The Adaptability of Fish Defense Mechanisms Against Anthropogenic Environmental Pollution**

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Enhancing our comprehension of the adverse effects on aquatic fauna due to environmental pollution necessitates a holistic approach. Employing passive sampling and subsequently determining defense mechanisms, stress indicators, and other biological effects *in vitro* in fish and benthic invertebrates can unveil the organism capacity to respond and cope with contaminants released into streams by municipal wastewater. The knowledge of pollution impacts on fish and their associated food chains can clarify the processes that allow fish to adapt to chemical exposure. Biochemical responses and histological changes in the organism were monitored in brown trout (*Salmo trutta m. fario*) restocked up and downstream of the sewage treatment plant (STP). Passive samplers (Speedisks and Silicone rubber sheets) were deployed at both sites to simulate the potential trans-membrane uptake of micropollutants into the organisms. The activity of biomarkers of oxidative stress and the catalytic activity of enzymes from the cytochrome P450 group involved in transforming foreign substances in the organism were determined in selected fish tissues. The results showed gradual adaptability to environmental pollution during the tested season from spring to autumn. The increased level of vitellogenin in the blood plasma of males indicated the impact of pollution on the balance of the endocrine system. Minor histopathological changes did not result from water pollution level but rather from a time-dependent growth variability of the tested individuals arising from other environmental factors. *In vitro* testing revealed the presence of compounds inhibiting progesterone receptors in passive samplers and benthos (*Ephemera* and case-making caddisflies), both at the control site and the site burdened with discharges of wastewater but not in the fish tissues. The study contributed to determining the response and adaptability of fish defense mechanisms against anthropogenically introduced chemical pollution into the aquatic environment. Since the study uses a holistic approach, this research may be considered environmental biomonitoring. The research was supported by the Czech Science Foundation (project No. 20-04676X) and by the Ministry of Education, Youth and Sports of the Czech Republic – project “CENAKVA” (LM2018099).

### **1.01.P-We015 Exploring the Potential of a Byproduct from Essential Oil Distillation: Ecotoxicity of Lavandin Hydrolates**

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During essential oil steam distillation, a significant volume of condensed water, known as hydrolates, is produced. Traditionally considered waste, hydrolates are now recognized for their antioxidant, antibacterial, and antifungal activities. This study assesses the ecotoxicity of hydrolates from *Lavandula x intermedia* Emeric ex Loisel, Lavandin, specifically the 'Super' (LS) and 'Grosso' (LG) varieties. Bioindicators *Daphnia magna*, *Vibrio fisheri*, *Allium cepa*, and *Eisenia fetida* were employed.

Aerial plant parts were manually harvested, and steam distillation in a stainless-steel distiller produced 5L of each hydrolate. Hydrolate composition, including total polyphenols, flavonoids, and proanthocyanidins, was analyzed using spectrophotometric techniques. Coumarin was quantitatively analyzed by HPLC-DAD, and volatile fractions were examined by GC-MS.

The Grosso variety contained 32 compounds, while the Super variety had 14, both including the main lavandin oil components: linalool, linalyl acetate, camphor, 1,8-cineole, and borneol. Monoterpenes constituted 96.1% in Super and 86.4% in Grosso, mainly in oxygenated forms.

Both hydrolates showed high ecotoxicity to terrestrial and aquatic organisms. In descending order of toxicity: *D. magna* > *E. fetida* > *V. fisheri* > *A. cepa*.

*D. magna* and *E. fetida* were the most sensitive, exhibiting mortality at low hydrolate concentrations: LC<sub>50</sub> = 0.23% for LG and 0.75% for LS in *D. magna*, and 0.82% and 0.84% for LG and LS, respectively, in *E. fetida*. *Vibrio fisheri* required slightly higher doses for luminescence loss: 1.60% and 1.07% for LG and LS. *A. cepa* root growth was inhibited at low hydrolate concentrations (EC<sub>50</sub> = 3.44% and 2.60% for LG and LS, respectively).

Pronounced toxicity in both indicators highlights the importance of considering the ecotoxic potential of natural products, crucial as interest grows in using these substances in agri-food, pharmaceutical, or cosmetic applications.

### **1.01.P-We016 Improving the Ecotoxicity Assessment of Waste from Mirror Entries: Evaluating the Suitability of a Biotest Battery**

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The European List of Wastes (2000/532/EC) contains 'mirror entries', wastes that can depending on their properties be classified as hazardous (when displaying at least one of 15 hazard properties) or non-hazardous. The hazard property 'ecotoxic' (HP 14) is assessed with a calculation approach based on the content of hazardous substances or using ecotoxicity tests with the respective waste sample or its eluate. For the testing approach, guidance at EU level is so far lacking. In several EU member states including Germany, recommendations for the ecotoxicological characterisation of wastes have been developed. The aim of the present project was to verify the recommendations published in 2013 by the German Environment Agency (UBA) and to develop suggestions for their further development.

To verify the suitability of the recommended test battery, ten waste samples from three mirror entries (10 09 09\*/10 09 10: flue-gas dust, 17 05 03\*/17 05 04: soils and stones, 19 10 03\*/19 10 04: fluff-light fraction and dust) were tested. Four of these wastes were classified as hazardous by their owners and six as non-hazardous. Waste sampling complied with the requirements of European (CEN/TR 15310) and German guidance (LAGA PN 98). Samples were sieved to a grain size of < 4 mm or, for the



*Arthrobacter* contact test, < 2 mm. The test battery consisted of three aquatic tests with waste eluate (acute daphnid test, algal growth test in microplates and luminescent bacteria test) and three terrestrial tests (earthworm avoidance test, growth test with higher plants and *Arthrobacter* contact test). Aquatic tests were repeated up to three times to evaluate reproducibility. According to the recommendations of UBA, a waste was classified as ecotoxic, if the EC<sub>50</sub> determined in at least one of the six tests was ≤ 10% eluate or solid waste.

For the aquatic tests, several test runs always yielded very similar results leading to the same classification. Algae and daphnids were more sensitive than luminous bacteria, and the aquatic tests tended to be more sensitive than the terrestrial tests. According to the test results, most wastes classified as hazardous by their owners were ecotoxic (HP 14). However, most wastes which had been classified as non-hazardous by their owners, were also ecotoxic. Based on the results of the experimental work, suggestions were developed how to update and further advance the German 'Recommendations for the ecotoxicological characterisation of wastes'.

#### **1.01.P-We017 The Road to Preliminary Identification of Techniques for Toxicity Monitoring and Chemical Key Environmental Issues**

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The oil refining process requires substantial water volumes for several purposes, such as cooling, steam generation, and processing of raw materials. After usage, some of the remaining water volumes are discharged. Industrial emissions should be assessed using Best Available Techniques (BAT), the latter according to the Industrial Emissions Directive (IED; 2010/75/EU) that also establishes what kind of parameters and how they should be monitored. In the upcoming years the Best Available Techniques Reference Document and its BAT Conclusions will be revised. In addition to chemical parameters, this is expected to include also effect-based monitoring. We have previously described an array of bioassays deemed suitable for the monitoring of refinery effluents, that are based on well-established protocols by OECD and ISO guidelines. In this pilot project, we aim at developing a testing program with the purpose of assessing the practical implementation challenges with the proposed assays at a selection of European refineries, to be performed in combination with parallel assessment of emissions levels. This complex project requires the measurement of several chemical parameters and toxicological endpoints, for which different sampling approaches and sample handling are needed. The adequate preservation of the sample offers additional challenges given the relatively scarce availability of local laboratories that have the capabilities to screen the samples for the selected bioassays. Large scale projects as this highlight the need of coordinated efforts between stake-holders, industry, NGOs and others to achieve common goals. Staying innovative and making the best-informed decisions is crucial to success in the ever-evolving landscape of Directives and other regulations, thus, our pilot project provides important insights as how to tackle complex problems at once advancing water quality assessments.

#### **1.01.P-We018 Strategies to address marginal and intermittent toxicity in effluent dominated systems.**

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As we continue to better manage water use within industrial facilities, effluent volumes can become reduced by water conservation. In these instances, chemical constituents in the wastewater maybe more concentrated, allowing more efficient treatment. However, less water may also be a concern for constituents that are recalcitrant to treatment. This issue is often magnified in effluent dominated receiving water systems. To overcome this limitation, as well as to account for the many effluents that contain mixtures of chemicals, whole effluent toxicity testing (WET) or whole effluent assessment (WEA) can more effectively determine whether effluent is toxic to aquatic organisms in its entirety, rather than by virtue of individual chemical components. Traditional WET or WEA methods use standard test species as fish, invertebrates, and algae as the analytical detectors to assess whether the effluents are unacceptably toxic even when the specific chemical composition is not immediately known. In effluent dominated systems, the regulatory trigger concentration can be at or nearly full-strength effluent. As a critical review, this paper will use case examples for the assessment of effluents to account for site-specific considerations of the discharge or receiving stream. One is faced with the challenge in effluent dominated systems to better understand if the results are due to effluent toxicity or whether the results are at least partially reflective of organism response variability. In these situations, the "but for question" needs to assess if the response in the effluent is due to chronic toxicity, or whether a necessary constituent that is present in the control water (or culture water) is missing in the effluent or does not provide the trace nutrients or food for adequate organisms' performance. This presentation will explore strategies to further evaluate effluents while employing novel assessment strategies, and still allowing the ability to resolve the specific chemical causing or contributing t the toxic effluent.

#### **1.01.P-We019 Why emission control technologies matter – An environmental perspective of catalyst and electrostatic precipitator application in biomass-based energy supply and their impact on aquatic systems**

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The increasing use of wood in residential heating is a major concern due to its impact on human health and ecosystems. The increasing demand for renewable energy sources further exaggerates this issue. To reduce the emissions from small-scale wood-fired heating appliances, the use of emission control technologies has become increasingly important. However, the efficiency of such technologies has been investigated mainly based on human health aspects, leading to significant neglect of ecotoxicological perspectives. In addition, the environmental risk assessment of atmospheric pollutants has focused primarily on particulate-associated toxicology, lacking effect data of the water-soluble fraction of wood smoke. Therefore, we applied two commercially available emission control technologies (ceramic foam catalyst (CAT) and electrostatic precipitator (ESP)) and investigated their impact on the acute (*Raphidocelis subcapitata*, *Daphnia magna*, and *Danio rerio*) and mechanism-specific toxicity (*in vitro* endocrine disruption, *in vitro* and *in vivo* EROD activity) of small-scale wood stove emissions. Beech wood was burned discontinuously in a small-scale wood stove, and water-soluble extracts of wood smoke (WSE) as well as organic extracts of deposited particulate matter (PME) were analyzed. Untreated WSE samples exerted pronounced acute toxicity to all investigated species (EC<sub>50</sub>: 0.080–0.876 m<sup>3</sup>/l). Endocrine activity (estrogenicity and anti-androgenicity) was detected in PME but not clearly in WSE. A similar trend was observed for the investigation of EROD activity. As expected, CAT treatment resulted in high mitigation efficiencies, however, ESP operation did not clearly reduce the observed toxicity of the wood-burning emissions. Non-target chemical analysis combined with hierarchical cluster analysis confirmed these observations. The data strongly suggest a temperature-dependent efficiency of the ESP as a function of gas-to-particle conversion. In addition, ESP significantly reduced PM emissions, but resulted in the formation of very coarse PM (i.e., PM<sub>>10</sub>). Overall, this study provides strong evidence that the use of CATs and, to a slightly lesser extent, ESPs in residential biomass heating appliances are promising measures to protect ecosystem health.

#### **1.01.P-We021 Progressing Short-term Methods for Estimating Chronic Marine Toxicity for Regulatory Use in the North-East Atlantic Region.**

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In the North-East Atlantic region, risk- and hazard assessment play an important role in the management and permitting of offshore chemicals, produced water and other wastewater discharges. Currently, assessments mostly rely on data from acute toxicity tests performed on a limited number of species. The application of acute tests potentially results in an overestimation of environmental risk due to the application of high assessment factors when deriving safe limits. Experience in some countries has shown that chronic toxicity testing can be an effective risk mitigating measure. However, due to their practical limitations and associated costs, chronic tests are not yet widely applied. There is a growing interest in the application of toxicity tests for the assessments of wastewater discharges both offshore and from land-based sites to coastal environments. Such tests might become essential to have appropriate and fit-for-purpose acute and chronic marine toxicity test protocols.

Under the umbrella of Offshore Energies UK (OEUK) a group of energy companies, chemical suppliers and industry associations have initiated a Joint Industry Project (JIP) that aims to pilot short-duration methods for estimating chronic marine toxicity tests for regulatory use in the North-East Atlantic region. The project aims to identify suitable methods for estimating chronic marine toxicity based on existing protocols and global practices and applicable to the management of both offshore and coastal discharges. The focus will be on protocols with short duration to reduce costs, optimize execution time and required logistics. Eventually the project aims to deliver a list of agreed and practical protocols that estimate chronic toxicity from which the endpoints are acceptable to all parties involved and includes tests that have been selected based on their availability and efficiency.

The project starts collecting and reviewing available short-term methods for estimating chronic toxicity. Selection of tests will be based on a comparison of available data on performance of such tests with the current acute tests. Piloting the methods on selected effluents and chemicals is foreseen within the project. The aim is to work with operators, chemical suppliers, testing laboratories, regulators, and their scientific advisors to develop a set of reliable short-term methods for marine chronic toxicity testing, ready for implementation upon project closure.

#### **1.01.P-We022 Impacts of Anthropogenic Stressors on Feral Fish: Biomarker Response in Locally Adapted and Translocated Brown Trout along the Holtemme River, Germany**

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Water pollution has emerged as a global problem due to the continually growing number and quantity of chemicals used by society. Key sources of pollutants to aquatic ecosystems are point sources such as wastewater treatment plants (WWTPs), as well as diffuse sources such as runoff from urban areas and agriculture. The resulting exposure to complex pollutant mixtures

have the potential to induce significant ecological effects, which are often not well understood. *In situ* studies, considering realistic environmental exposure, are required to better characterize these ecological effects. This study aimed to investigate impacts of multiple chemical stressors on feral brown trout (*Salmo trutta*) by comparing biomarker response patterns of locally adapted and translocated individuals along the Holtemme River, Germany. While the upper reaches of the river are relatively unimpacted representing natural conditions, downstream areas are subjected to cumulative human impacts, accompanied by increased levels of chemical pollution. In brief, five groups of brown trout were exposed for 21 days at three sites along the river. Respectively, one fish group was caught and caged at all three sites (reference, urban and downstream of a WWTP) and represent the locally adapted fish. For translocation experiments, two additional groups were caught at the reference site and translocated to the anthropogenically impacted downstream sites (urban and downstream of the WWTP). For a better understanding of exposure impacts, multiple biomarkers in kidney were examined due to its role in excretion and metabolism of pollutants. The measured biomarkers are indicative for xenobiotic metabolism (glutathione *S*-transferase, benzyloxy-4-trifluoromethylcoumarin-*O*-debenzyloxylase and ethoxyresorufin-*O*-deethylase) and oxidative stress (glutathione reductase and catalase). Preliminary results indicated stronger alteration of enzyme activities in translocated fish compared to locally adapted. In contrast to locally adapted individuals, translocated fish experienced elevated levels of stress due to a changed environment. After molecular sex determination, only marginal differences in the effects between sexes were identified. At the location downstream of the WWTP, female fish exhibited increased enzyme activities, which may well be explained by sex-related variations in sensitivity to pollutants discharged from the WWTP.

### 1.01.PC Advancing the Use of Effect-Based Approaches for Water Quality Assessment

### 1.02 Alternatives to Animal Testing for Ecotoxicity Assessments: Exploring Approaches and Avenues for the Future

#### 1.02.T-01 Importance of internal concentrations and toxicokinetics to classify modes of action of organic chemicals in the zebrafish embryo (*Danio rerio*)

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There is a pressing demand to use new approach methodologies and powerful computational modeling approaches for chemical risk assessment. Environmental risk assessment at present is typically based on chemical exposure in water. Aqueous concentrations are only a proxy for the internal concentrations of a compound in the organism, which are the biologically effective doses and vital to know to interpret the toxic effects. There are various approaches available that predict the steady-state uptake of chemicals into the organisms and bioconcentration factors based on the physicochemical properties of the test compound. The zebrafish embryo (*Danio rerio*) has widely investigated to establish such predictive models and understand the mechanistic basis of the toxic effects of chemicals. However, these approaches are not the perfect predictors for internal concentrations as they do not consider specific toxicokinetic processes, e.g., biotransformation and active transport, which can substantially affect the bioconcentration of certain chemicals. Additionally, experimental internal concentration data are rare and most of them are only based on predicted bioconcentration factors and physicochemical properties of the chemical. Therefore, we measured steady-state concentrations of 61 polar and non-polar compounds by determining their internal concentrations in the ZFE after 96 hours of exposure at one sublethal exposure concentration and compared them to a mass-balance model (MBM). We found a close relationship with measured concentrations typically in the 1:1 range for 82 % of the study compounds. We identified 11 of 61 chemicals showing a significantly lower internal concentration than predicted. For some of these chemicals, various transformation products could be identified via UPLC-HRMS and their relative contribution to the total internal concentration indicates a significant transformation of the parental compound. In case of 7 of the 11 substances with internal concentration deviating from the model, biotransformation and/or specific transport had an influence on the classification of the specificity of the respective chemical, as this caused the membrane concentration to decline below the baseline toxicity range. This highlights the importance of toxicokinetics on the correct assessment of the specificity of the observed effect caused by a chemical.

#### 1.02.T-02 Establishing a 24-Hour, Microplate-Based, Transcriptomics Assay for Rainbow Trout Embryos

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There is interest in the development of early-life stage (ELS) tests with fish embryo models that are high-throughput and can generate transcriptomics point of departure (tPOD) values. The objective of this study was to establish a high-throughput toxicity test for larval fish that could simultaneously generate tPOD values, and basic mortality data. We based our pilot method on recent efforts by U.S. EPA researchers to establish a larval fathead minnow high throughput transcriptomics assay. Briefly, rainbow trout alevins (1-2 day post-hatch) were placed in individual wells (24-well plates) and exposed for 24 hr to 12 different concentrations of OECD 249 test chemicals (and other chemicals of interest), including a negative control (fish water or 0.5% DMSO) for 24 hours. Test concentrations were based on a tapered design which included an LC50 push zone (10- and 100-fold higher than estimated LC50 from the U.S. EPA EcoTox database), transcriptomics point of departure (tPOD) zone (six concentrations below the LC50 range on a half log<sub>10</sub> basis), and baseline zone (10- and 100-fold lower concentrations).

Using this design, the exposure for 3,4-dichloroaniline was: 1000, 320, 100, 32, 10, 3.2, 1, 0.32, 0.1, 0.032 and 0.01 mg/L (covering 7 orders of magnitudes). Presently, we have tested 25 distinct chemicals using this design. LC50 values have been generated for 3,4-dichloroaniline (58.5 mg/L), copper sulfate (0.3-0.8 mg/L), glyphosate (150 mg/L), malathion (0.6 mg/L), acetaldehyde (41.2 mg/L), 4-fluoroaniline (243 mg/L), ethanol (18.2 g/L), 6-PPD quinone (5.6 ug/L), aniline (1026 mg/L), pentachlorophenol (0.6 mg/L), lindane (3.4 mg/L), 4,6-dinitro-o-cresol (0.3 mg/L), dimethyl sulfoxide (6.5%), caffeine (153.2 mg/L), sodium dodecyl sulfate (14.5 mg/L), methylmercury (0.5 mg/L), ethinylestradiol (>100 ng/L), thiamethoxam (>300 mg/L), permethrin (>3 mg/L), allyl alcohol (>30 mg/L), dibutyl phthalate (>300 mg/L), metformin (>10000 mg/L) and guanylurea (>5000 mg/L); preliminary LC50 values are provided in parentheses. Repeated studies of copper sulfate yielded consistent LC50 values. Work is underway to produce transcriptomics data for these samples using EcoToxChips and UPXome, with the goal to be able to derive iPODs. Coupling all of this together, these results will provide a foundation towards establishing a novel testing platform for chemical and environmental risk assessment.

### 1.02.T-03 Using Endpoint Response Patterns to Aid Interpretation, Support Read-Across and Reduce Testing with the Amphibian Metamorphosis Assay

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Read-across is regarded as a strategy to reduce animal testing for chemical safety assessment. The OECD Conceptual framework (CF) for screening and testing of endocrine disruptors (EDs) includes read-across as relevant non-test information that may be available at Level 1. The amphibian metamorphosis assay (AMA; OECD 231) is a short-term *in vivo* assay (CF Level 3) that is recommended in the ECHA-EFSA Guidance for the identification of EDs as necessary/adequate to demonstrate the absence of thyroid-mediated endocrine activity. However, in the EU context, with hazard-based cut-off criteria for EDs, the very conservative decision logic of this assay carries to significant regulatory consequences. Small changes in hind limb length at only one time point are sufficient to warrant a positive result, which may lead to unnecessary animal use through higher tier testing. We have taken a holistic approach to interpret AMA studies by looking at endpoint response patterns and applying this to an anonymised dataset comprising publicly available AMA data conducted for the US EPA EDSP, OECD validation assays, and proprietary data on plant protection active ingredients. We categorised endpoints as informing on: (i) overt toxicity, (ii) growth or (iii) development. A total of 94 independent studies were encoded for agonist-type or antagonist-type responses. Responses to the thyroid hormone agonist tetraiodothyronine and perchlorate (competitive inhibitor of the sodium-iodide symporter) were regarded as model pro-thyroidal. Anti-thyroid substances and remaining substances were sorted according to similarity of response patterns across growth and development parameters. This dataset was then interrogated for phenotypic patterns associated with indication, structural class, or known toxicological mode of action. Our findings indicate that very few plant protection actives exhibit response patterns comparable to known thyroid axis disruptors and for several classes, patterns indicative of systemic toxicity (inhibited growth and development, no compensatory changes in thyroid histopathology) are clearly apparent. This approach offers the potential for preliminary assessment of active substances through read-across to inform choices on data generation supporting evaluation of thyroid-mediated activity in non-target organisms. In turn this may support greater use of *in vitro* or other non-animal testing approaches, with consequent reductions in animal use.

### 1.02.T-04 An In Vitro Disposition Model for the Fish Cell Assay According to OECD Test Guideline 249

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OECD TG249 describes a cytotoxicity assay with the rainbow trout gill cell line, RTgill-W1, as an *in vitro* alternative for the acute fish toxicity test. The cell assay was found to be a good predictor for *in vivo* toxicity, if the measured chemical concentration is considered instead of the nominal concentration. Binding of the chemical to the well plate, to medium and to cell components reduce the bioavailable concentration and hence the apparent sensitivity of the assay. To this day, measurement of the free chemical concentration is lower in throughput than the toxicity measurement itself. Hence, the *in vitro* disposition of chemicals is often predicted. Most *in vitro* disposition models have been developed for assays with human cells, which are typically cultured in medium containing 5-10% fetal bovine serum (FBS). The FBS acts as “buffer” and keeps the freely dissolved concentration in the medium fairly stable, when chemicals are taken up into cells and the plastic of the plates. In contrast, in TG249, the cells are exposed to the chemicals in a minimal medium without FBS, which leads to a depletion of the freely dissolved concentration. Therefore, we conceived a novel *in vitro* disposition model suitable for lean cell culture media.

To describe the *in vitro* disposition, a kinetic mass balance model was implemented as a nested model consisting of three compartments: medium, cells and plastic. Cells consist of three subcompartments (water, proteins, lipids), which are considered in instantaneous equilibrium. The initial adsorption to the plastic is fast but the internal diffusion is very slow and hence the overall loss to plastic is time-dependent and was implemented as a kinetic process. Uptake kinetics into cells was assumed to be similar as for human cell lines and was described by a first-order kinetics one-compartment model.

The model is provided as an Excel spreadsheet and as an R script. We applied it to predict the *in vitro* disposition of 199 neutral and ionized organic chemicals ( $-3 < \log K_{ow} < 7$ ) tested in the RTgill-W1 assay. We predicted that for 72% of chemicals, the free concentration was >30% of the nominal concentration. For more hydrophobic chemicals, the free concentration was up to 1000 times lower. This indicates the importance of adjusting for *in vitro* disposition, as for those chemicals the *in vivo* effect concentration would be predicted 1000x too high, leading to a less protective effect concentration.

## 1.02.P Alternatives to Animal Testing for Ecotoxicity Assessments: Exploring Approaches and Avenues for the Future

### 1.02.P-Mo001 Single and Combined Effects of Sertraline and Polyhydroxybutyrate Nanoplastics to Amphibians: An In Vitro and In Vivo Approach

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Plastic pollution is a major factor impacting wildlife, with amphibians being an especially vulnerable group. Nanoplastics (NPs) gain relevance due to their widespread and particular characteristics, including large surface area and reactivity. Polyhydroxybutyrate (PHB) emerges as a bio-based plastic, however, the understanding of its environmental impact, particularly regarding its nanosized particles, remains limited. Sertraline, one of the most consumed antidepressants, is expected to be present in the aquatic environment due to patient excretion, inefficient wastewater treatment, and improper disposal. The objectives of this work were to assess the effects of PHB nanoplastics and sertraline, in single and combined exposure, towards two aquatic early life stages of the model organism *Xenopus laevis*. Additionally, in vitro assays were conducted to understand the suitability of two cell lines as non-animal alternatives. To attain these objectives, both embryos and tadpoles of *X. laevis* were exposed for 96-h to increasing concentrations of sertraline and PHB-NPs, as well as combined (only for tadpoles) exposures, where the endpoints assessed were mortality, occurrence of malformations, heart rate and growth (body length and body weight, the later endpoint only for tadpoles). On the other hand, 72-h cytotoxic assays were conducted with two cell lines derived from *X. laevis* (A6 and XTC-2), where the cell viability was assessed through the MTT and resazurin viability assays. Our results suggested high tadpole sensitivity to sertraline (96-h LC<sub>50</sub>: 0.45 mg L<sup>-1</sup>), compared to embryos (96-h LC<sub>50</sub>: 2.58 mg L<sup>-1</sup>), while concentrations of PHB-NPs between 0.01 and 100 µg L<sup>-1</sup> did not show statistically differences in the monitored endpoints. Furthermore, A6 and XTC-2 cell lines were less sensitive to sertraline with 72-h LC<sub>50</sub> of 5.05 and 3.78 mg L<sup>-1</sup>, respectively, while the opposite was observed for the PHB-NPs. On *in vitro* assays PHB-NPs and sertraline co-exposure showed both examples of antagonism and synergism were observed. Our results suggest cell lines, though not being more sensitive than tadpoles and embryos to sertraline, seemed to be good alternatives for animal testing, promoting fast and reliable preliminary assays, and allow a more precise selection of concentration ranges to be tested at later phases of environmental risk assessment, consequently reducing the number of organisms used in animal testing.

### 1.02.P-Mo002 Screening Chemicals Using High-Throughput Phenotypic Profiling (HTPP) in Two Zebrafish Cell Lines

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High-throughput phenotypic profiling (HTPP) combines Cell Painting, where chemically treated cells are labeled with fluorescent probes to quantify changes in cell morphology, alongside a propidium iodide (PI)-based cell viability assay used to identify cell cytotoxicity. One of the goals of ongoing HTPP work is to expand the range of cell lines tested to encompass a wide array of target organs and origin species. The current study applied HTPP to two previously untested zebrafish cell lines: one from adult liver (ZFL) and one an embryonic fibroblast (ZEM2S). Two individual chemical sets were tested in 8-point half-logarithmic concentration-response format. The first, was a set of 23 reference chemicals known to cause morphological changes in a variety of cell types, and the second consisted of 42 chemicals covering a diverse range of uses such as drugs, industrial chemicals, and chemicals used in foods/consumer products. DMSO-solubilized chemicals were dispensed onto cells plated in 384-well format, and sampled after a 24-hour exposure. Treatments defined as cytotoxic by the cell viability assay were excluded from subsequent analyses. Cell Painting data were analyzed using pipelines designed to normalize data to vehicle control and dimensionally reduce data by calculating Mahalanobis distances across all features, as well as within individual categories of features. Curve fitting was performed across global, category, and individual feature level. A phenotype altering concentration (PAC) was determined as the minimum benchmark concentration between global and category-level endpoints. Active chemicals were defined as those having a PAC value below the highest tested concentration. Most of the 23 reference chemicals were active in both cell lines: 19 for ZFL, 17 for ZEM2S. Of the 42 chemicals in the larger test set, approximately half were active: 24 in ZFL, 21 in ZEM2S, with an overlap of 17 chemicals. We have previously demonstrated that HTPP is an efficient and informative approach for bioactivity screening of environmental chemicals in human-derived cell models. Here we have adapted the HTPP approach to cell types from zebrafish, a vertebrate, non-human model organism widely used in toxicology as a step to increasing the species diversity of this NAM tool. These data are foundational for future HTPP screens across cell types and model organisms. *This abstract does not reflect USEPA policy.*

### 1.02.P-Mo003 Ecdysone Receptor Agonism Adverse Outcome Pathway Validation for Insect-Specific In Vitro Assay Development

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With the growing population, competitive economy and climate change, the assessment of Environmental Risks is essential for handling the pressure of a high variety of chemicals entering the environment. Historically, Environmental Risk Assessment (ERA) protocols were limited to testing a small number of endpoints on mostly *in vivo* exposures of a small number of “representative” model species as a proxy to the entire ecosystem. To address obvious shortcomings of these approaches and to address a changing regulatory and ethical landscape towards reducing or eliminating animal testing, New Approach



Methodologies (NAMs) represent a shift towards a more mechanistic ERA, including the use of Adverse Outcome Pathway (AOP). This approach is operationalised predominantly by *in silico* and *in vitro* testing assays. For such mechanistic-based ERA, the development, evaluation and qualification/ validation of *in vitro* assays is imperative, representing critical steps in advancing environmentally sound and effective risk assessment.

The aim of this project is to provide empirical evidence of an assay based on Ecdysone Receptor Agonism AOP towards establishing an *in vitro* cell-based assay. Ecdysone, an insect hormone, is released in pulses that trigger developmental transitions by initiating a cascade that begins with binding to the Ecdysone receptor. According to the proposed AOP, the ecdysone levels in the organism are reduced at a specific moment, which allows later genes in the moulting cascade to be expressed, enabling the organism to complete the moulting process. This is a highly conserved pathway across arthropod species, therefore, chemicals with the ability to interfere with Ecdysone receptor can cause incomplete moulting and subsequent mortality. Using *Drosophila melanogaster* and its embryonic (S2) cell line as a model system, we aim to characterise and quantify the molecular and cellular event cascades underlining Ecdysone receptor mediated toxicity in invertebrates and explore the applicability of the S2 cell line as an *in vitro* assay. Preliminary results show that upon exposure to ecdysone, S2 cells respond in a similar way as the initial steps of the moulting cascade that is only completed once ecdysone is removed from the system as is seen *in vivo*.

If successful this approach could be deployed for other relevant pathways and endpoints to ensure a robust environmental protection without the reliance on further generation of invertebrate data.

### **1.02.P-Mo004 Predicting Acute Fish Toxicity with the RTgill-W1 Assay (OECD TG 249): Putting it into Regulatory Practice**

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Determination of acute fish toxicity is a key regulatory endpoint for environmental hazard assessment globally. The Fish Gill Cell Line (RTgill-W1) cytotoxicity assay provides a non-animal alternative that has been validated and adopted as an OECD Test Guideline (TG 249). However, adoption of an OECD TG does not equate to regulatory acceptance. As highlighted in the recent ECHA NAM workshop report, gaining regulatory acceptance relies on demonstrating the use of, increasing confidence in, and understanding the limitations of New Approach Methodologies (NAMs). Adaptation of regulatory frameworks was also recognised as being crucial. Encouragingly, the draft REACH revision includes a proposal to replace short-term fish toxicity testing with the RTgill-W1 assay (TG 249) or the fish embryo test (TG 236).

We previously demonstrated the applicability of the RTgill-W1 assay to fragrance chemicals covering a broad range of physicochemical properties and diverse chemistries. From this study, we developed a regression equation translating the *in vitro* EC<sub>50</sub> to the *in vivo* LC<sub>50</sub>. According to TG 249, the lowest EC<sub>50</sub> based on geometric mean measured concentrations among three cell viability endpoints is used as a direct estimate of the predicted LC<sub>50</sub>. Here, we compare the two approaches for the fragrance chemicals of the previous study and 10 additional chemicals. Both approaches can predict LC<sub>50</sub> values accurately, yet the regression approach is more conservative.

We will also present a number of case studies, demonstrating our use of the RTgill-W1 assay in regulatory applications. This includes REACH registrations; not only > 10tpa substances but also, in anticipation of the REACH revision for increased information requirements for low tonnage substances, a number of Annex VII substances. The applicability to complex mixtures is explored as is the use of the TG 249 assay as a standalone assessment or in combination with QSAR and/or to support read-across.

Only mutual acceptance of the results by regulatory bodies around the globe guarantees that no animal tests need to be conducted. Thus, we will present one case study submitted to the Japanese authorities where we used the RTgill-W1 assay supported by QSAR (ECOSAR, KATE) to demonstrate that the primary metabolite observed in the ready biodegradability study was less toxic than the parent. On this basis we proposed to waive the acute fish toxicity test of the metabolite in order to avoid unnecessary animal testing.

### **1.02.P-Mo005 Breaking the silos: Towards a holistic approach of animal-free human and environmental health safety assessments of cosmetics**

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Historically, human and environmental health safety assessments have operated as distinct entities, often in silos. The need to

move away from animal testing for chemical safety purposes stresses the importance to identify key strengths and share learnings from each domain while highlighting intricacies and opportunities towards a holistic animal-free safety assessment.

Human health safety assessments already utilize many animal-free methods within frameworks for several endpoints. This is particularly the case for the cosmetics sector with its long-standing move away from animal testing and the complete EU animal testing ban by 2013. Here, exposure led next-generation risk assessment (NGRA) frameworks are being proposed to demonstrate an absence of human health effects for cosmetics without using animals.

To eliminate animal testing for environmental safety, both animal-free methods to understand effects (e.g. aquatic toxicity) and refined exposure (including fate) methodologies are needed. However, in comparison to human health, only few animal-free approaches exist and there is simultaneously, less funding and expertise available, hindering rapid evolvments. However, the possibility of using invertebrate models to generate environmental safety data can be of benefit also for human health, as a proxy for fully functioning organisms.

While acknowledging differences, such as protection goals, a holistic animal-free approach could build on:

- Understanding biological relevance such as conserved targets, pathways and traits driving species sensitivities;
- Leveraging advancements in technologies such as microphysiological and high-throughput screening methods;
- Tackling regulatory needs such as internal exposure concentrations and endocrine disruption;
- Fostering collaborations among stakeholders including regulatory bodies, validation organizations, industry and test method developers;
- Discussing regulatory frameworks to ensure alignment between human and environmental safety assessments where appropriate;
- Demonstrating the utility of animal-free methods via case studies.

This poster will present a thought-starter from the International Collaboration on Cosmetics Safety (ICCS) to actively engage with SETAC on how to foster collaborations across human and environmental health domains, revolutionizing the way we understand potential impacts of chemicals without using animals.

#### **1.02.P-Mo006 Lessons learnt from XETA OECD TG248 for implementation of NAMs in regulatory assessment of endocrine activity.**

*David Du Pasquier, Andrew Tindall and Gregory F. Lemkine, Watchfrog S.A., France*

In 2019, the *Xenopus* Eleuthero-embryo Thyroid Assay (XETA, OECD TG 248), marked a significant milestone as the first eleuthero-embryo method validated by the OECD for assessing thyroid activity.

The XETA utilises *X. laevis* eleutheroembryos to detect modulation of thyroid signalling by thyroid active chemicals. The assay is transcription-based and uses a transgenic tadpole line expressing Green Fluorescent Protein (GFP) under the control of a promoter regulated by TH. As an “alternative method”, the XETA is considered as a new approach methodology (NAM). NAMs encompass methods that predict effects by identifying molecular, cellular, and tissue changes, aiming to reduce the use of animals.

An important step for the application of the TG248 was the publication of an annex to the “Guidance for the identification of endocrine disruptors in the context of EU regulations” describing how to include the XETA in the assessment strategy of the ECHA/EFSA Guidance.

This is of great interest considering the implementation of NAMs as it followed a mechanistic approach for a comprehensive coverage of the targets of endocrine mechanism. Following this guidance, when no thyroid mediated adversity was observed in mammals and the XETA is negative then “the endocrine activity for the T-modality for non-target organisms other than mammals is considered sufficiently investigated and the Endocrine Disruptor criteria are not met.”

Since the XETA was implemented 99 active ingredients were evaluated for the T modality by the authorities. Ten were eligible for replacing the AMA by a XETA.. Considering that a typical AMA requires 452 animals, we could estimate that the implementation of the XETA as defined by ECHA and EFSA guidance saved 4520 animals. The actual number of biocides and pesticides requiring an evaluation for ED properties is estimated to 475 pesticides and 297 biocides, resulting in a total of 396 264 amphibians required to perform AMAs for the T modality. If we estimate that 10% of the AMAs will be replaced by a XETA, this will avoid the use of approximately 40 000 amphibians.

Combining eleuthero-embryos with data from in vitro single mechanism assays could provide comprehensive coverage of the targeted modes of actions along the endocrine signalling pathway. In particular, this would speed up the process of endocrine assessment of large numbers of chemicals/cosmetic ingredients which would more rapidly protect wildlife from endocrine active chemicals.

### **1.02.P-Mo007 Advancing Thyroid Hormone Disruption Assessment: Integration of Adverse Outcome Pathway Network and Cross-Species Relevance of In Vitro Bioassays**

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The applicability of data derived from alternative in vitro bioassays in risk assessment is often hindered by the inherent oversimplicity of these assays. Nevertheless, significant improvements in the applicability can be achieved through the incorporation of the Adverse Outcome Pathway (AOP) concept. This concept establishes a link between molecular initiating events (MIEs), assessed in vitro, and adverse outcomes (AO) at the individual or population level. The EU H2020 ERGO project adopts the AOP framework to develop a battery of in vitro bioassays for screening chemicals with potential endocrine-disrupting properties on the highly conserved thyroid hormone (TH) system, facilitating the acquisition of cross-species relevant data. A cross-species AOP network for TH disruption is under development, providing a basis for prioritizing MIEs within the bioassay battery.

We have established and optimized a battery of bioassays focusing on prioritized endpoints related to thyroid hormone disruption. The cross-species relevance of selected MIEs is supported by comparing the primary structure of target proteins across animals using the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) online tool from the US EPA. Bioassays assessing the effect of thyroid-disrupting chemicals (TDCs) on iodide uptake by thyroid cells, mediated by the Na<sup>+</sup>/I<sup>-</sup> symporter (NIS), utilize a stably transfected human cell line overexpressing NIS and a rat thyroid cell model. Iodide uptake levels are detected spectrophotometrically using Sandell-Kolthoff reaction (SK). Transport of TH is evaluated by fluorometric assessment of TH displacement from its plasma transporter, transthyretin (TTR), by TDCs. TH metabolism is addressed using a cell model overexpressing deiodinase (DIO3), which is a key factor in maintaining controlled levels of TH during the developmental processes. The DIO3 inhibition by TDCs was detected using SK. These assays, tailored for screening prioritized chemicals, showcase the utility of the newly developed bioassay battery for high-throughput screening of chemicals. The results enable the characterization of their thyroid hormone-disrupting potential and identification of the most relevant MIEs. Based on SeqAPASS analysis, data from the established human cell-based bioassays are deemed relevant to most vertebrate species. The project has received funding from the EU H2020 research and innovation programme under grant agreement No. 825753

### **1.02.P-Mo008 Frequency of Malformations in Zebrafish Embryos Exposed to Metals**

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The zebrafish *Danio rerio* has been proposed as a test organism to evaluate the effects of chemical compounds. Embryos of this species are currently used, because their response is comparable to that of adult fish. In Mexico, the use of zebrafish is not stipulated in current legislation, despite being a useful alternative to replace fish bioassays. The objective of this study was to determine the toxic effect of 10 metals, that have been detected in concentrations greater than 50 mg/L in systems in the Valley of Mexico, to determine their sensitivity and propose their use in monitoring studies. Initially, bioassays were carried out lasting 24 hours, seven metal concentrations were tested in triplicate (0.01, 0.1, 1.0, 5, 10, 20 and 40 ppm) for Cd, Cr, Cu, Hg, Pb and 6.25, 12.5, 25, 50 and 100 ppm for As, Mn, Ni, V and Zn. The LC<sub>50</sub> was determined after 48 hours of exposure using the Probit method. Subsequently, another test was carried out lasting 72 hours with 2 sublethal concentrations (LC<sub>1</sub> and LC<sub>10</sub>), where the frequency of embryos with malformations was determined. The toxicity of the metals based on the LC<sub>50</sub> was (from highest to lowest toxicity): Hg > Cu > Pb > Cr > Cd > As > V > Ni > Mn > Zn. Deformed larvae were obtained in concentrations between 0.003 and 49.08 mg/L of metal. The highest frequencies of deformed embryos were recorded in the tests with Cr and Pb. Tests with embryos evaluating the frequency of malformations are a quick and economical option for carrying out biomonitoring studies.

### **1.02.P-Mo009 Assessing the Applicability Domain of Fish Embryo Acute Toxicity (FET) Test as Alternatives of Fish Acute Toxicity Test, Utilizing Time-to-death Data in FET Test**

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Acute fish toxicity data is globally utilized for chemical risk assessment purposes in compliance with various regulatory frameworks. However, concerns have been raised regarding the unnecessary suffering and significant fish fatalities resulting from this test, as it involves exposing fish to high concentrations of a test chemical to induce mortality. The fish embryo acute toxicity test (FET; OECD TG 236) has been emerged as a potential alternative test method to the acute fish toxicity test (OECD TG 203), as it is considered a non-protected life stage under certain animal welfare regulations. Although it has been reported that many chemicals exhibit a generally similar toxicity value between the two test methods, there are cases that discrepancies are observed in toxicity values between the two test methods. Therefore, adopting FET test as an alternative method for the fish acute toxicity testing presents challenges, primarily due to the uncertainty surrounding its applicability domain.

In this study, we aimed to define the applicability domain of the FET test as an alternative method through a novel approach that involves categorizing test chemicals based on time-to-death data obtained in the FET test. The FET test encompasses both the embryo and eleutheroembryo stages, effectively bridging the gap between cell-based assays and whole fish testing.

Therefore, we hypothesized that narcotic chemicals were expected to cause death at the early developmental stage within two days of fertilization, while chemicals acting via a specific mechanism, such as those targeting specific tissues, were anticipated to cause death at the eleutheroembryo stage, where ontogeny have concluded.

Our results indicate that pharmaceuticals and pesticides with specific targets exhibited later onset of lethality during testing except for lovastatin. Meanwhile, most general chemicals, initially predicted not to have a specific target, displayed early onset of lethality. These results indicate that time-to-death data obtained from FET testing might distinguish chemicals explained by toxicity acting on specific targets from other toxicity such as baseline (narcotic) toxicity. Furthermore, we suggest that the result based on this chemical categorization approach could be one of the crucial pieces of evidence to define applicability domain of the FET test as an alternative method.

### **1.02.P-Mo010 Development of an In vitro Marine Fish Bioassay for Characterizing Environmental Impact of Industry Development in the Arctic**

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The ongoing changes in the Arctic, such as rising temperatures and diminishing sea ice, are likely to introduce new and/or expansion of current industrial activities, including oil, gas, and aquaculture operations. There's a pressing need to understand how these human activities affect the Arctic environment and wildlife, focusing on the transmission, bioaccumulation and effects of ecologically-relevant pollutants. To address this effectively, reproducible, cost-effective and ethically, New Approach Methodologies (NAMs), using in vitro methods were implemented to quickly assess the toxicity of compounds of concern. The main goal of the study is to develop and implement an *in vitro* testing system originating from the Arctic fish, Atlantic Halibut (*Hippoglossus hippoglossus*), examining how environmentally relevant chemicals affect the liver (hepatocyte) cells, and evaluate their use in prediction of environmental consequences. This goal will be achieved by multiple tasks: 1) Development and optimization of primary hepatocyte cell isolation, cell characterization (morphology, physiology and structure), culturing and exposure of Halibut to model compounds (e.g. Copper,  $\beta$ -Estradiol, 2,3,7,8-Tetrachlorodibenzodioxin (TCDD)); 2) Assessing hepatocytes sensitivity and responsiveness of key indicators of toxicity such as detoxification, hormonal disruption, and antioxidant responses; 3) Finally experimental evaluation of selected substances that are particularly relevant to the Arctic environment. The preliminary results display that the primary hepatocytes are diverse, as they consist of more than one cell population. The majority of cells ( $\varnothing$ 12-16  $\mu$ m) were complex, containing large amounts of intracellular lipid droplets, followed by a less complex, but bigger cell type ( $\varnothing$  ~21  $\mu$ m), identified with a light microscopy. Upon exposure to the model compounds copper and TCDD, an acute toxic effect was observed in both cell membrane integrity and metabolic activity, with the latter being more susceptible. Overall, the use of *in vitro* methods such as halibut primary hepatocytes is a promising proxy for *in vivo* testing and a potential tool in future hazard and (next generation) risk assessment of Arctic contaminants. This work was supported by the EXPECT Project (#315969) funded by the Research Council of Norway.

### **1.02.P-Mo011 Are both the water and the solvent control required in fish early-life stage toxicity tests?**

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This project investigates whether it is possible to use a solvent control alone when a solvent is required in fish early-life stage (FELS) toxicity studies. Use of only one control would substantially reduce the number of fish used in studies requiring a solvent.

The project compiled a FELS toxicity test database including solvent and water controls and concentration-response data to determine whether there are systematic differences between the water and solvent controls. For each response variable, the distributions of control data (means, between- and within-replicate variances) for water, solvent and pooled controls were investigated. The authors used FELS concentration-response data to investigate the impact of control choice on the NOEC and EC<sub>10</sub>. Using computer simulations, the authors determined the potential impact of using only the solvent control on the EC<sub>x</sub> estimation and NOEC values. The simulations covered the observed ranges of variability and concentration-response shapes for each type of response across three species (Fathead Minnow, Sheepshead Minnow, and Rainbow Trout). The study explores all response variables required in FELS toxicity studies including hatching success, time to hatch, time to swim-up, larval survival, morphological and behavioural abnormalities, fish length and fish weight at study end. Furthermore, the study explores both the selection of statistical model and model averaging for EC<sub>x</sub> estimation.

According to the simulations, for the solvent dimethylformamide (DMF) and Fathead Minnow, use of the solvent control maintains 80% power to detect 10% effects in length and weight and 20% effects in hatch/survival with lower false positive rates than other control choices. The analyses of FELS concentration-response data are also presented. Altogether, there is preliminary statistical evidence to support omitting the water control in FELS studies using a solvent.

The views, conclusions, and recommendations expressed in this presentation are those of the authors and do not necessarily represent the policies or positions of their affiliated organisations.

## **1.02.P-Mo012 Fins to Future: Assessing the Role of Acute Fish Toxicity Testing in Risk Assessments of Plant Protection Products in the EU and the US**

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Both the European Union (EU) and the United States (US) are actively pursuing initiatives to reduce the use of vertebrate animals in toxicity testing. Under Commission Regulations (EU) No 283/2013 and No 284/2013 in the EU, and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) in the US, the fish acute toxicity test is required for plant protection product (PPP) registration and ongoing authorization. With alternative non-vertebrate methods like the fish embryo toxicity (FET – OECD TG 236) test and the rainbow trout gill cell line assay (RT gill-W1 – OECD TG 249) available, it is important to assess the concrete value, that the fish acute toxicity test contributes to the overall environmental risk assessment.

To address this, we presented a critical review of aquatic risk assessments for EU-registered active substances (a.s.) at SETAC Dublin in 2023. To broaden the scope and enhance the relevance of this topic, an additional analysis was conducted on the approach employed in the US, complementing the EU risk assessment. The objective of this data review was to determine how frequently the acute fish toxicity endpoint, as opposed to other aquatic organism toxicity endpoints, drives the risk for a.s. in the aquatic environment across the globe.

Our evaluation covered 57 a.s. registered in the US (under Bayer ownership) and 232 a.s. registered in the EU. The findings revealed that a minimal percentage of aquatic risk assessments was driven by the acute fish endpoint for both the US and the EU risk assessment approach (less than 1% in the EU after refinement, and approximately 10% in the US; the US data set will be expanded with further registered a.s.).

This evaluation suggests significant potential for minimizing vertebrate testing while still ensuring protectivity for the aquatic environment, including fish. Moreover, alternative tools such as the FET, RT gill-W1 and in-silico tools (e.g. QSARs) can offer additional insights into fish-specific toxicity and will be addressed in future investigations. This research underscores the potential for conducting aquatic risk assessments for PPPs without relying on acute fish toxicity studies. In addition, this work aims to increase regulatory acceptance of alternative tools for the registration of PPPs in the individual regions and to support the 3Rs principle.

## **1.02.P-Mo013 A Network for Alternative Methods for ecological safety assessment of chemicals (ecoNAM)**

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ecoNAM is a platform to facilitate international information exchange about animal alternatives for ecological safety assessment of chemicals. Our aim is to foster cooperation between government, academia, industry, Non Governmental Organisations, research centers and other stakeholders involved in the research, development, and application of alternative ecological hazard and risk assessment tools and methods. ecoNAM will facilitate the exchange of knowledge, data, and expertise to promote synergies and collaboration related to advances in ecological alternative methods on a global scale. We are gauging interest and resources needed for such a network and will determine the next steps and a feasible path forward. This poster describes ecoNAM and how it has development since its inception in 2023. The following critical needs, purpose and goals of ecoNAM are identified as:

**CRITICAL NEED:** There are existing networks for animal alternatives focused on human health endpoints, but similar groups do not exist for ecotoxicity testing. There is significant interest from different initiatives and organisations discussing NAMs but in a poorly coordinated way which needs addressing. There is a need to create a global network of multi-sector partners working to advance alternatives to ecotoxicity testing and to bridge the divide between ongoing initiatives in the human health arena.

**PURPOSE:** The purpose of ecoNAM is to promote global international cooperation between government, academic, industry, NGOs, research centers, and other stakeholders involved in research, development, and application of alternative ecological risk assessment methods.

**GOALS:** ecoNAM has the following goals:

- Facilitate exchange of knowledge, data, and expertise to promote collaboration related to advances in ecoNAMs on a global scale.
- Strategically prioritize needs and identify resources within the ecoNAM community.
- Promote the uptake and application of ecoNAMs.
- Develop training and education materials.

The Network for Alternative Methods for ecological safety assessment of chemicals can be found at the following link [www.econam.org](http://www.econam.org)



### **1.02.P-Mo014 *Caenorhabditis elegans* Life Cycle Tests as Screening Tools to Unveil the Underlying Adverse Effects of Chemicals in Reproduction**

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Chemical substances from various sources enter wastewater treatment plants and end up in the environment. Pesticides and pharmaceuticals are usually some of the most found chemical substance classes found in waterbodies and soil. The ubiquitous distribution of these substances raises worldwide concern regarding their potential deleterious effects on non-target organisms and humans. Adverse effects in reproduction output are some of the most concerning ones, where some effort is needed to accurately pinpoint the moment or process where the disruption occurs. In order to provide some insights, *Caenorhabditis elegans* figures out as an outstanding animal model to tackle this problem due to its well-known lifecycle and effectively suitable within a One Health approach.

For this study, four chemical substances, found worldwide in waterbodies, were selected: the insecticides cypermethrin and flupyradifurone, the herbicide MCPA, and the over-the-counter pharmaceutical diclofenac. Different life stages of *C. elegans* life cycle were assessed for each substance to unravel the effects in distinct reproductive stages/processes. The embryotoxicity/neonatal toxicity was evaluated by assessing the hatching rate of exposed *C. elegans* eggs; the developmental toxicity was evaluated by exposing individuals from L1 larvae to egg-laying adults and assessing the total reared offspring; the transgenerational effects (mother-to-progeny) were evaluated by measuring the hatching rate of unexposed eggs obtained from exposed adults. Our results show that all substances affect the reproductive output of *C. elegans* by exerting effects in one or more life stages/reproductive processes. Cypermethrin was the most toxic chemical regarding the observed developmental effects, whereas MCPA figured out as the most toxic considering the neonatal and transgenerational effects.

The present study's targeted approach provides supporting evidence on the unexpected reproductive effects of chemicals unrelated to their mode-of-action. The results obtained also highlight the potential of *C. elegans* to fill in some gaps in the development of Adverse Outcome Pathways contributing to a more realistic hazard prediction which can be extended to other organisms and, ultimately, humans and animals, within the One Health approach.

### **1.02.P-Mo015 Initiatives for the regulatory acceptance of New Approach Methodologies, how does it all connect?**

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The last years were marked by a period of regulatory change, driven by important policy initiatives related to the European Green Deal, EU Chemical Strategy for sustainability and the Pharmaceutical Strategy for Europe. In response to the 2021 Parliament Resolution to 'Accelerate the transition to Innovation without the use of Animals in Research, Regulatory Testing and Education' and European Citizens' Initiative (ECI) 'Save Cruelty-Free Cosmetics', the European Commission has initiated a series of actions aimed to progress the transition to complete animal-free testing for EU chemicals legislation (e.g., REACH, Biocidal Product Regulation, Plant Protection Products Regulation and human and veterinary medicines). In parallel, academia and industry are driving the transition towards NAMs application through their research and development. Despite the considerable progress in the scientific development of NAMs, barriers to their regulatory uptake remain. These barriers have become a subject of research themselves. Major findings from an empirical study include that next to the technical barriers that have been described in the past and are a continuous subject to academic discussions, there are a range of social barriers that manifest in a resistance to the technological transition. The focus on social and people-based factors distinguishes the study from narrower scientific debate which has tended to focus on the maturity of the next generation of chemical risk assessment. The findings highlight the nuances to the subject that need to be understood in order to determine the root cause of the perceived delay in regulatory uptake of NAMs. In response to the European Citizens Initiative, the EU Commission has initiated a series of activities aimed at collating the community's expertise by designing a roadmap on the transition to NAMs and creating opportunities for engagement and generation of consensus. A detailed analysis of the ongoing and future initiatives was translated into a stakeholder map that can be used to identify major stakeholder and opinion leaders' positions. With an increasing understanding of what the crucial next steps towards the replacement of animal testing are, what challenges remain? How do our efforts need to be coordinated in this time of transition? Here we present an analysis of the most important developments in EU policy and global stakeholder driven activities that should be considered moving forward.

### **1.02.P-Mo016 Prediction of the In Vivo Acute Toxicity of Agrochemicals to Fish with the In Vitro RTgill-W1 Cell Line Assay**

Casten Lange Dr<sup>1</sup>, **Gunnar Schmidt<sup>2</sup>**, Lea Weinisch<sup>1</sup>, Kristin Schirmer<sup>3</sup>, Stephan Fischer<sup>4</sup>, Jennifer Fitzgerald<sup>5</sup> and Maike Habekost<sup>1</sup>, (1)BASF SE, Germany, (2)BASF SE, Research Triangle Park, United States, (3)Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland, (4)aQuaTox-Solutions GmbH, Switzerland, (5)Eawag - Swiss Federal Institute of Aquatic Science and Technology, Switzerland

The RTgill-W1 cell line assay (OECD 249) provides an animal-free alternative to *in vivo* fish acute toxicity tests (e.g. OECD 203 & 236). Testing for acute fish toxicity is an integral part of the environmental risk assessment for chemicals in Europe and other parts of the world. Dozens of fish are required for a single study with mortality being the primary endpoint. The studies also require large amounts of test material and produce remarkable volumes of contaminated water. Acute fish toxicity is

mainly driven by an exposure of the gill epithelium to dissolved chemicals. The gills constitute the primary uptake site for water-borne substances, where unspecific effects (loss of membrane integrity or metabolic activity) might occur. On a cellular level, those adverse effects lead to death of epithelial cells and a loss of function of the gill epithelium. Affected fish suffer from respiratory failure, ultimately leading to death.

The RTgill-W1 cell line was derived from rainbow trout (*O. mykiss*) gill epithelium. Cells are treated in a 24-w plate with 6 concentrations for 24 h and EC<sub>50</sub> values are generated with cell viability assays (metabolic activity, lysosomal disruption, cell membrane integrity) and are used to predict *in vivo* fish toxicity.

The RTgill-W1 cell line assay has been intensively tested on chemicals with various modes of action (MoA) and a broad range of physico-chemical properties and toxicity. However, data on plant protection products are scarce. Therefore, we conducted the RTgill-W1 assay with a selection of 11 active substances from the agrochemical sector with different indications and MoA over a broad concentration range (0.05 µg/L to 100 mg/L). A strong correlation between *in vitro* EC<sub>50</sub> values and existing *in vivo* LC<sub>50</sub> values (rainbow trout, 96h) with fold-change differences below 10 were observed.

With the current dataset, we provide evidence for a strong predictivity of the RTgill-W1 assay for a set of agrochemicals that have been underrepresented in published data. The data shows that the RTgill-W1 assay is applicable for agrochemicals with different indications and MoA and over a broad concentration range. The results support the potential of the *in vitro* assay to replace *in vivo* fish acute toxicity tests for agrochemicals. We also intend to raise regulatory acceptance for the use of the RTgill-W1 assay by adding another supportive piece to a convincing accumulation of data that has been published for chemicals from various sectors.

### **1.02.P-Mo017 Animal-free In Vitro Assessment of Receptor-Mediated Endocrine Activity Including Phase-1 Metabolism**

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Substances interfering with the endocrine system (endocrine disruptors) are present in the environment in complex mixtures and pose a risk to organisms and entire ecosystems, even at low concentrations. Therefore, evaluation of the endocrine activity of environmental samples or individual substances is of central (eco-)toxicological importance.

One possibility for evaluation is the application of standardized, cell-based *in vitro* tests according to OECD test guidelines 455 and 458, in which estrogen and androgen receptor agonists and antagonists can be detected after the addition of the sample. Nevertheless, these *in vitro* test methods have the following disadvantages: 1) Cell-based *in vitro* tests are conducted with the addition of animal-derived components like fetal bovine serum (FBS) to promote cell growth. However, the replacement of FBS in cell assays can be advantageous for scientific reasons in context of reproducibility (FBS having a high batch-to-batch variability, interaction with test substances, microbial contamination) and animal welfare reasons. 2) *In vitro* tests are usually performed with cell lines that do not fully reflect the metabolic capabilities of a whole organism. Thus, a lack of full metabolization capacities may lead to an over- or underestimation of the endocrine effects of a sample.

In this study, the methods, according to OECD TG 455/458, should be improved by adapting two of the OECD-relevant cell lines, ER- $\alpha$ CALUX® and AR-CALUX®, to animal-free culture conditions. Therefore, commercially available as well as self-developed animal-component free cell culture media were successfully used for the cultivation of both cell lines. After adaptation, the cell lines will be tested for their sensitivity to the respective reference substances to verify the suitability of the adapted cells for an OECD 455/458-compliant test procedure. In addition, both assays are to be supplemented with an external human-derived or biotechnologically produced biotransformation system (S9 homogenate) to evaluate the endocrine activity of model substances after metabolism.

Overall, the endocrine effects without and with S9 homogenates and without or with adaptation to animal-free cell culture conditions will then be compared to assess the assay adaptation and their (beneficial) impact on risk assessment. Thus, the optimized test procedure might improve assay reproducibility and strengthen *in vitro* assays as an alternative to animal experiments.

### **1.02.P-Mo018 Challenges in the Application of New Approach Methodologies (NAMs) to Assess the Ecological Hazard of Unknown, Variable, Complex and/or Biological substances (UVCBs)**

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New approach methodologies (NAMs) are increasingly being used for chemical hazards to support regulatory decision-making without animal testing. However, information is still scarce to demonstrate the use of NAMs in the ecological assessment of Unknown, Variable, Complex and/or Biological substances (UVCBs). This poster will provide an overview of the challenges in the application of NAMs to UVCBs and suggest recommendations to promote their implementation with this important group of substances. The complex nature of UVCBs poses technical and biological challenges in the application and regulatory acceptance of NAMs. UVCBs are considered difficult-to-test substances with technical implications in the experimental design

of the in-vitro tests, including, but not limited to, the preparation of solutions, dosing, maintaining exposure concentrations, and analytical chemistry measurements. Moreover, UVCBs are usually non-intentional mixtures of chemicals, and the complete chemical structure or the specific molecular formula is unknown. Due to the lack of this information and the variable composition of multi-constituents UVCBs, the use of in-silico and QSARs models, grouping-based approaches (e.g., red-across), and the understanding of mechanisms/modes of action may be limited. Regarding biological challenges, the lack of in-vitro tools (e.g., cell lines from different tissues and species) specific for the hazard assessment of aquatic species, questions the biological coverage of the data. Moreover, the extrapolation from in-vitro to in-vivo but also from one species to the whole population needs to be considered. To validate the use of NAMs and facilitate the decision-making process, the justification for the selection of UVCB substances to build case studies needs to be clearly . Also, standard criteria and protocols for validation need to be identified to guarantee the reproducibility, robustness, and reliability of the tools and results. Mapping roadmaps to identify gaps, reviewing currently available data, improving current models and QSARs, developing specific UVCBs ecotoxicological methods and tools, integrating technologies such as omics, building case studies based on weight of evidence approaches, understanding the interpretation of the data and defining validation criteria, are key areas to promote the use of NAMs to assess the ecological hazard of UVCBs.

### **1.02.P-Mo019 Advancing Chronic Fish Testing: Needs and Challenges for Alternative Approaches**

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Chronic fish toxicity is a key parameter for hazard classification and environmental risk assessment of chemicals. With an increased focus on the development and application of new approach methodologies (NAMs), there is a need to ensure that an effective path forward includes considerations for animal reduction, refinement, and replacement (3Rs) while also focusing on how new science can improve our ability to protect the environment from potential fish chronic effects. To address this issue, the Health and Environmental Sciences Institute (HESI) workshop on “Alternatives to Chronic Fish Toxicity Testing” was held in Paris, France on October 25-26, 2023, attended by 35 multi-sector scientists from 12 different countries, and a satellite workshop was held in Louisville, Kentucky on November 12, 2023, attended by 16 multi-sector scientists from 3 different countries. This presentation is a summary of some of the key messages representing new lines of thought regarding alternatives to chronic fish ecotoxicity testing. Based on discussions at the workshops there was a general shift away from the concept of one-for-one replacement and towards an integrated approach for chronic fish testing linking various approaches and lines of evidence. In addition, there was interest in developing a menu of approaches to prioritize traditional chronic fish *in vivo* testing such that it is only conducted when truly needed to fill data gaps. In discussions, attendees emphasized combining various approaches, both *in vivo* and NAM-based, to address different aspects of chronic fish toxicity (including bioaccumulation and endpoints associated with specific modes of action, e.g., endocrine) and to reduce uncertainty in risk assessments. Whole effluent toxicity testing in North America was identified as a potential key area where implementation of NAMs could reduce vertebrate use; however, it was decided that at this time effluent testing should be its own effort that takes learnings from the output of the workshop. A repeated need was for a concerted effort to develop a coordinated network of individuals working within the ecoNAM space to be established with an initial goal of mapping out ongoing method developments and data collection efforts. Based on the discussion from the two workshops, specific topics/projects will be considered by expert work groups for further development and investigation.

### **1.02.P-Mo020 Importance of the positive control in the Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay (OECD TG249)**

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In 2021, the OECD Test Guideline (TG) 249 was approved as the first cell line-based assay for environmental chemical risk assessment. As it no longer requires fish, it comprises a true animal alternative. It measures acute toxicity exerted to RTgill-W1 cells, using three fluorescent indicator dyes to indicate cell metabolic activity (using a resazurin dye, such as alamarBlue<sup>®</sup>), cell membrane integrity (based on the esterase substrate, CFDA-AM), and lysosomal membrane integrity (using Neutral Red). RTgill-W1 is a permanent cell line established from a rainbow trout (*Oncorhynchus mykiss*) gill. It was purposefully selected for test development as the gill epithelium is considered one of the most vulnerable tissues in acute fish toxicity testing, as performed, e.g. according to OECD TG 203. The RTgill-W1 cell line assay therefore has the potential to significantly reduce and replace application of one of the most commonly but also most severe fish toxicity tests carried out in product development and hazard assessment for chemical/product registration.

One key feature of the OECD TG 249 is that it contains, aside from negative controls, a prominent assay performance (positive) control. More specifically, with each run of the assay, a full concentration-response analysis is done with a reference chemical, 3,4-Dichloroaniline (3,4-DCA). Effective concentrations causing a 50% decline for each of the fluorescent indicator dyes (EC50 values) are derived, which must lie within a pre-defined range as specified in the TG. We have gathered a large and steadily growing set of 3,4-DCA control data. Based on this experience, it has become evident that this positive control is indeed very stringent, probably the most stringent in any of the biological OECD TG. Failures usually concern the impact on cell metabolic activity and have empirically been linked to stresses exerted on the cells, such as temperature and handling stress. On the flip side, this means that meeting these positive control criteria provide high confidence in the cell viability data

measured for chemicals alongside in the same run. We will present the most comprehensive data set to date and discuss the importance of this stringent quality control.

### **1.02.P-Mo021 Screening Naphthenic Acid Contamination in the Athabasca Oil Sands Region in Chicken LMH 3D Spheroids**

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Oil extraction in the Athabasca Oil Sands Region (AOSR) generates large volumes of waste, called tailings, that are stored in mined out areas called tailings ponds. These tailings are toxic largely due to the presence of naphthenic acids (NAs) and there is concern about seepage into the surrounding tributaries. The objective of the present study was to determine the concentration of NAs and *in vitro* bioactivity of extracts derived from passive samplers deployed in tailings ponds and surrounding natural wetlands in the AOSR. NA concentrations were determined using liquid chromatography tandem mass spectrometry. Chicken LMH cells, cultured as 3D spheroids, were dosed with extracts and bioactivity was measured using cell viability, ethoxyresorufin-O-deethylase (EROD), and gene expression assays. Gene expression changes were determined using the chicken ToxChip qPCR array which contains 48 toxicologically relevant genes. Here, we present preliminary results for the surrounding wetland area extracts. As expected, higher levels of NAs were measured in passive sampler extracts from wetlands closer to oil sands activity compared to those further away. There were no effects on cell viability for any of the wetland extracts; however, several genes were dysregulated on the chicken ToxChip qPCR array. The dysregulated genes were associated with various pathways including xenobiotic metabolism, lipid homeostasis, and immune function. Next steps include the measurement of EROD activity to evaluate CYP1A expression in the natural wetlands, followed by determining NA concentrations and resulting bioactivity of six tailings pond extracts. Ultimately, we aim to demonstrate the use of a non-animal screening method for environmental monitoring in an ecosystem of concern in Canada.

### **1.02.P-Mo022 Evaluation of Positive Control Substrates for the OECD319 In Vitro Rainbow Trout Bioaccumulation Estimation Studies**

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The OECD 319 guidelines for the determination of *in vitro* intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP, OECD319A) and S9 sub-cellular fraction (RT-S9, OECD319B) were published in 2018. The studies are intended as a means of estimating the potential for bioaccumulation of chemicals in fish without the need for an *in vivo* fish bioconcentration factor (BCF) study (OECD305). They can be used in cases where it is not possible to run the BCF study, for example, ethical reasons such as cosmetic use, to reduce animal usage or it is not technically feasible. They may also be used to provide early screening information about bioconcentration potential or metabolism.

Since the release of the guidelines there have been discussions around the most applicable positive control substrates for the experiments. Positive controls are used to ensure the metabolic viability of the test system used (S9 or hepatocytes). A positive control usually should typically result in a minimum transformation of 20% with ideally 10% remaining unchanged by the terminal time-point of the experiment. This can present a challenge in the case of particularly high or low turnover as the positive control could be entirely transformed or remain unchanged at the end of the experiment. To validate the OECD319 study at our facility we have tested several different substrates that have the potential to be used as a convenient positive control. These can be matched to the clearance of the chemical under investigation.

This poster presentation will summarise the results from the testing of several different potential positive control substrates for both the RT-HEP and RT-S9 assays.

### **1.02.P-Mo023 Application of OECD TG 249 for assessment of the toxicity of metal oxide nanomaterials**

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The use of *in vitro* models to assess the toxicity of nanomaterials (NMs) is a valuable tool generating essential information that can be used in a number of approaches, for instance for designing higher tier *in vivo* test or in read-across procedures. OECD Test Guideline (TG) 249 allows the assessment of acute toxicity of chemicals in fish using the RTgill-W1 rainbow trout gill cell line. The test is validated for a range of organic toxicants and it demonstrated a high correlation with *in vivo* fish acute toxicity. However, its applicability to NMs has not yet been evaluated. This study aimed to apply this TG to NMs, using some metal oxide NMs and comparing the results with those obtained with other fish cell line and with fish.

The following NMs were studied: CuO, 15-20 nm, (PlasmaChem); CuO, <50 nm, (Sigma-Aldrich); ZnO NM-110, 42 nm; ZnO NM-111, 34 nm; TiO<sub>2</sub> NM-101, 6 nm; TiO<sub>2</sub> NM-104, 20 nm; and CeO<sub>2</sub> NM-212, 33 nm, (JRC repository). For obtaining stable dispersions the Nanogenotox protocol was applied (<https://safenano.re.kr/download.do?SEQ=175>). First, the usefulness

of commercial L-15 (Thermo Fisher Scientific Inc.) and L-15ex, without fetal bovine serum, were compared. Cells exposed to 3,4-Dichloroaniline (3,4-DCA) served as a positive control, whereas those maintained in L-15ex with bovine serum albumin were the negative control. RTgill-W1 ( $35 \times 10^4$  cells/well) cells, were seeded in 24-well plates following the TG249 scheme. A triple cytotoxicity assay (Alamar Blue, CFDA-AM and neutral red) was performed according to TG249. Potential interferences of NMs with the cytotoxicity assays were assessed.

Compared to L-15, the L-15ex medium decreased cell viability by 7-15%, making it acceptable for this test. For TiO<sub>2</sub> and ZnO there was a characteristic interference, which should be taken into account when interpreting the results. The EC<sub>50</sub> values of 3,4-DCA in RTgill-W1 cells were within the limits described in the TG and compared with those obtained with RTL-W1 cells ( $28 \times 10^4$  cells/well) that due to a decrease in sensitivity were out of limits stated in the TG. The results obtained in fish cell lines were similar to those collected from acute toxicity studies in fish. Future studies will set the best procedures to apply TG249 to NMs.

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### **1.02.P-Mo024 Towards next generation risk assessment for Environmental Health: how PARC Work Package 5 is contributing**

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The European Partnership for the Assessment of Risks from Chemicals (PARC) is a unique concept of partnership that brings together risk assessors, authorities and the scientific community to implement innovations in testing and chemical risk assessment into regulation. In particular, work package 5 (WP5) “Hazard Assessment” contributes to provide data to fill gaps in knowledge on poorly characterised contaminants and the consideration of new approaches and methods of hazard assessment, promoting the use of innovative methods and tools and contributing to moving towards next generation risk assessment. It also explores the improvement of mechanistic understanding of toxicity by analysing all available data and applying systems toxicology approaches and taking into account adverse outcome pathways (AOPs) and to improve modelling approaches such as PBK modelling.

WP5 focuses on hazard assessment for human and environmental health. For this purpose, two projects are dedicated to environmental toxicity assessment by filling data gaps of the substances prioritised for this first period of PARC: natural toxins and BPA alternatives. One of the projects under WP5 investigates the toxicity of naturally occurring toxins on aquatic organisms, specifically invertebrates and microalgae, so that the data generated could be a basis to set EU guidelines to achieve good environmental status for marine and freshwater environments. The other project focuses on the investigation of the potential adverse effects of certain individual substances and “real-life” mixtures of BPA alternatives on non-mammalian organisms belonging to different taxa. Moreover, the project serves as a platform for the development of new technologies, such as *in silico* and modelling tools for the grouping of substances and read-across, and advancing invertebrate and vertebrate models as new approach methodologies (NAMs) including e.g., (meta)genomic, transcriptomic, high-content analysis microscopy, high-resolution mass spectrometry, and human stem cell technology to assess (eco)toxicological endpoints such as non-genotoxic carcinogenicity, (developmental) neurotoxicity, immunotoxicity and endocrine disruption (ED), specifically thyroid hormone system disruption and metabolic endocrine disruption.

It is our aim to disseminate WP5 work in order to invite dialogue between the scientific community and regulators, promoting the integration of results into policy.

### **1.02.P-Mo025 What is the most sensitive cellular scale bioassay to multiple chemicals with different mode of action?**

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*In vivo* and *in vitro* tests with unicellular organisms and cell lines show promise for evaluating diverse chemicals in cellular-scale systems. While ideal for high-throughput screening in effect-directed analysis, uncertainties persist regarding the sensitivity and selectivity of cellular-scale assays across classical *in vivo* and *in vitro* tests for identifying specific chemical classes based on their mode of action. To identify the optimal cell-based assay for sensitivity, we assessed three unicellular organisms from different kingdoms (plant, fungi, and bacterial) *in vivo*, alongside five *in vitro* cell lines, using 21 chemicals. These chemicals, ranging from antimicrobials to metals, were selected for their diverse modes of action and common occurrence in European wastewater and surface waters. *In vivo* tests included growth inhibition assays on the algae *Raphidocelis subcapitata*, the yeast *Saccharomyces cerevisiae* and bacteria *Escherichia coli*. *In vitro* tests, using MTS cell viability as an endpoint, were Zebrafish embryonic fibroblast cell line (ZF4), Hamster ovary cell line (AREcoscreen-GR-KO-M1), Mouse hepatocellular carcinoma (DR ecoscreen) and the Human breast carcinoma cell lines (VM7luc4E2 and MCF7AREc32), representing cells from different vertebrate species. Algae were the most sensitive tests across both *in vivo*

and *in vitro* tests, producing concentration-response curves for 19 out of the 21 chemicals tested within the testing range. Algae was particularly responsive to antimicrobials, herbicides, fungicides and the metals Cu and Zn. Surprisingly, algae surpassed *E. coli* in sensitivity to four of five tested antimicrobials, and was more sensitive to the three fungicides tested than *S. cerevisiae*. The mammalian cell lines were the most sensitive *in vitro* tests, responding to 10-14 of the 21 chemicals within the range tested, hence, the overall hierarchy of sensitivity in terms of detection frequency was as follows: algae > human cells > rodent cells > fish cells > *E. coli* > yeast. Comparing the EC<sub>50</sub> algae were 1- >100 fold more sensitive to 10 of the 14 common chemicals, whereas VM7luc4E2 was many folds more sensitive to the remaining four common chemicals. Therefore, the divergent yet heightened sensitivity of these two tests to a wide range of chemicals establishes them as a valuable complementary pair for screening bioactive substances in environmental samples of unknown composition.

Keywords: cellular scale bioassay, *in vivo*, *in vitro*; sensitivity.

### **1.02.P-Mo026 Identification of ToxCast bioassays associated with developmental and reproductive toxicity and their application to mechanism-based chemical screening: Case study with biocidal chemicals under K-BPR**

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With the introduction of new chemical regulations, the demand for developing high-throughput screening (HTS) methods to prioritize regulatory chemicals is growing. The EPA ToxCast database, one of the largest HTS databases available, provides extensive biological coverage across diverse molecular targets, encompassing all major protein subfamily groups. To accelerate the use of ToxCast bioassays in a regulatory context, it's essential to establish links between molecular toxicity endpoints and regulatory endpoints. This study addressed this need by investigating ToxCast bioassays associated with developmental and reproductive toxicity, utilizing 499 overlapping ToxCast chemicals with animal toxicity data from ToxRefDB. Firstly, correlation analysis using Point-biserial and Pearson's methods revealed that 92 bioassays exhibited a significant correlation ( $p$ -value < 0.05) with animal toxicity data. Secondly, among these assays, 31 were identified as engaged in teratogenic mechanisms, including processes related to sex hormone metabolism, oxidative stress, extracellular matrix, angiogenesis, and more. Employing the selected ToxCast bioassays, we screened 109 biocidal chemicals regulated under K-BPR that lacked available developmental and reproductive toxicity data. This screening identified 10 chemicals prioritized based on their activities in selected ToxCast bioassays, suggesting their potential to induce developmental and reproductive toxicity. These chemicals are commonly found in preservatives, disinfectants, repellents, pesticides, and rodenticides. Our approach shows promise as a mechanism-based categorization method, particularly when *in vivo* toxicity data are unavailable.

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### **1.02.P-Mo027 Transcriptomic points of departure for solvent and positive controls in rainbow trout and human cell lines**

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Ethical concerns and high costs associated with animal testing have prompted widespread interest in developing more efficient non-animal based alternative testing strategies. There has also been increased interest in using molecular data in risk assessment for benchmark dose response analysis and the derivation of transcriptomic points of departure (tPOD). The objective here was to optimize and establish a high throughput *in vitro* transcriptomic-based toxicity test system that couples rainbow trout RTgill-W1 and human Caco-2 and HepG2 cell lines with QIAGEN's UPXome ultraplex technology so that the combined test system can derive tPODs. Library preparation was first optimized with unexposed RTgill-W1 cells to ensure good quality libraries as verified on an Agilent Bioanalyzer. Subsequently, cells were exposed for 24 h to eight concentrations of solvent (dimethyl sulfoxide; DMSO) and positive controls (3,4-dichloroaniline; DCA – RTgill-W1 or hydrogen peroxide; H<sub>2</sub>O<sub>2</sub> – Caco-2) from which libraries were prepared and sent for sequencing. Exposures were based on the OECD test guideline 249. The advantage of this workflow is that cell lysis, conducted in microplates, allows for plate-based reverse transcription directly on the lysate without isolating RNA from each sample. During reverse transcription, a unique sample index for each sample is incorporated into the cDNA which allows for pooling of the cDNA and simplified library preparation than traditional methods. Thus, libraries can be prepared from multiple 96-well plates in one workday, and hundreds of samples can be sequenced in a single lane. The strongest concentration-dependent transcriptomic response was observed upon exposure to DMSO with over 1,000 gene BMDs across all three cell lines. Exposure to DCA resulted in the least gene BMDs and the highest tPOD (7 mg/L), whereas to H<sub>2</sub>O<sub>2</sub> resulted in the most sensitive tPOD in HepG2 (0.07 mM). Many DEGs were involved in processes including stress response, cell signaling, motility and survival, and phase-II drug metabolism including heat shock proteins, selenoprotein P and angiopoietin-like 4. With increased interest in the use of transcriptomics in toxicity testing, these types of studies can help inform future test guidelines and identify appropriate candidates to be included as controls. Further exposures to evaluate the performance of this platform with diverse environmental samples and chemicals of regulatory concern are ongoing in several case studies.

### **1.02.P-Mo028 Microbial Assays for Risk Assessment to Evaluate Toxicity of Chemicals and Environmental Samples**

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### Benefits of microbial bioassays

Bacteria are good candidates for ecotoxicity testing because of number of factors that include small size, rapid growth rate and ease of handling. The use of bacterial bioassays to assess toxicity is well established, and is attributed to the rapidity of results and convenience of use.

Single species bacterial tests have been in common use across a wide range of industry and environmental applications for many years.

The best known tests make use of the naturally luminescent bacteria *Aliivibrio fischeri* (previously known as *Vibrio fischeri*). The best known tests are based on the rationale that exposure to toxins disrupts the bacterial respiratory processes, which in turn results in a reduction in light output.

MARA and LumiMARA bioassays with the incorporation of a genetically diverse multiple strains combination have significantly advanced microbial assays from single species utilisation.

Bacterial testing is generally based around assessment of the acute toxicity response of a single marine species. In contrast, testing using both MARA and LumiMARA gives a measure of acute and chronic toxicity, encompassing marine, freshwater and terrestrial species of prokaryotes (bacteria) and additionally a single-celled Eukaryote (yeast).

**MARA:** The MARA test assesses toxicity by measuring the growth of ten bacterial species and one yeast, following 18 hours of incubation in the presence of a concentration gradient of the test sample. As a eukaryote inclusion of a yeast species gives a valuable additional dimension to test results.

**LumiMARA:** LumiMARA assesses toxicity on the basis of the reduction in luminescence of 11 species of bacteria following exposure to a concentration gradient of the test sample. The test includes nine marine and two fresh water species from a variety of habitats. Two of the strains are of *A. fischeri*, one of which is an equivalent to NRRL B-11177 referenced in ISO 11348-3.

Application in the oil and gas industry: The Oslo and Paris Commission (OSPAR) Recommendation 2012/5 requires contracting parties, of which the UK is one, to implement a risk-based approach for the management of produced water from offshore oil and gas installations. MARA and LumiMARA bioassays are recommended as part of the UK's Department for Energy Security and Net Zero (DESNZ) preferred approach to achieving this

### 1.02.P-Mo029 Utility of QSARs and zebrafish embryos toxicity results in a simplified assay and in the OECD TG 236 to predict the fish acute toxicity of fragrance ingredients

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Fish acute toxicity data are part of the required information in the process of chemicals' registrations around the world and are used for environmental hazard and risk assessments. Prior the regulatory process, chemicals companies screen new substances in the phase of development to understand their toxicity profiles. To that end, cost-effective and reliable tools to predict fish acute toxicity, without using animals, should be used. Fish acute toxicity results from tests (zebrafish, OECD TG 203) used for regulatory submission of fragrances ingredients were used to verify the predictivity of the outcome of QSAR models (ECOSAR and iSafeRat) and the results from the standard Fish Embryo Test (OECD TG 236) and from a simplified version. More than 30 fragrance ingredients belonging to various chemical classes (alcohol, esters, aldehydes, etc) and with a wide physico-chemical profile (water solubility from 0.01 to 1000 mg/L, vapour pressure from < 0.1 to > 100 Pa) were tested in a simplified OECD TG 236. In the case of volatile substances precautions were taken to avoid losses and cross contamination. The 96h LC50 were obtained from the simplified assays (8 concentrations, DMSO as vehicle, 15 embryos per experimental condition, 5 embryos per well in 24 well-plates, no analytical measurements). The 96h LC50 were based on the standard lethality indicators for fish embryos. The results from both embryotoxicity tests (the simplified-assay and the OECD TG 236) showed similar trends, with an average factor of 2 difference in the 96h LC50 values. In most cases, the OECD TG 236 was more conservative. There was little difference between the zebrafish juveniles LC50 values (OECD TG 203) based on measured and nominal concentrations, reflecting that the exposure concentrations had been well maintained, except for the substances with the highest vapour pressure. In the embryotoxicity simplified assays, the toxicity was also underestimated ( $V_p$  from 10 to 400 Pa). The juvenile fish toxicity predictions by ECOSAR are more conservative than the predictions by iSafeRat, while the estimations of the embryotoxicity was under-estimated in 1/3 of the cases, especially when it comes to very volatile substances. After the complete analysis of this unique dataset, we aim at finding a recommendation for a suitable approach for reliably screening new chemicals for their fish acute toxicity and potentially to use that information in a regulatory context.

### 1.02.P-Mo030 Optimization of Experimental Conditions in Microphysiological systems (MPS) and Evaluation of Hepatotoxicity of Triphenyl Phosphate (TPHP) in HepG2 Cells

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Over the recent decades, the reliance on mammalian animal testing has been customary for evaluating toxicity of chemicals. However, existing animal testing models have inherent limitations such as interspecies differences, ethical issues, and difficulty in efficiently assessing large numbers of chemicals, so there is a strong need to shift toxicity assessment paradigm away from the current dependence on animal testing. As one of notable paradigms within the framework of New Approach

Methodologies (NAMs), the implementation of Microphysiological Systems (MPS) has attracted great attention. The intricately engineered systems, designed to simulate a similar environment to the human body by integrating fluid flow and cell co-culture technologies, have evolved since the introduction of the 'Lung-on-a-chip' in 2010. Despite the technological advancements in the development of MPS, cases of its use in the toxicity assessment of chemical substances are limited and have not been sufficiently discussed. In this study, we applied human-derived cell line, HepG2 was cultured in a MPS to evaluate the hepatotoxicity of an organophosphorus flame retardant, triphenyl phosphate (TPHP). In addition, we discussed how the MPS can be used in the toxicity evaluation of chemical substances in the future by comparing toxicological consequences of TPHP obtained from plate culture, an existing cell culture model.

We optimized the cell numbers and flow rates of our self-produced SperoMimic MPS and showed that LDH and ALT activities that could not be observed in traditional static culture models can be observed in MPS under optimized experimental conditions. We therefore demonstrated that MPS is a much more effective approach for identifying chemicals with the potential for hepatotoxicity. Additional research will follow to upgrade the MPS to allow for longer exposures. This allows us to better assess the incidence of hepatotoxicity or liver disease due to chemical exposure, serving as a true alternative to existing animal models

### **1.02.P-Mo031 Digital Patterns for the Detection and Prediction of Chemically Induced Endocrine Disruption in Fish (DiMEP = Digitale Muster Erkennung und Prädiktion)**

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Endocrine-active substances can endanger human health and the environment by impairing essential endocrine-controlled functions, such as the development and reproductive capacity of organisms. In 2018, the assessment of endocrine-disrupting properties (ED) of chemicals was included in European chemicals legislation under REACH and the Plant Protection Product regulation.

Currently, there are 434 compounds under investigation for ED, and more than 1000 are listed as potential candidates. For the aquatic environmental hazard assessment of substances with suspected ED, *in vivo* mechanistic bioassays, such as the Fish Short Term Reproduction Assay (FSTRA, OECD 229 & 230), are conducted with 80-120 fish per test. Therefore, more than 100,000 fish will be used for testing in the next few years for nearly 1500 chemicals. This number of sacrificed animals will further increase in the future as the ED hazard assessment applies to a wide variety of compounds (e.g., plant protection products, biocides, industrial chemicals).

To reduce the number of animal tests for ED assessment in aquatic vertebrates, in accordance with the 3R principles, the UFZ (Helmholtz-Zentrum für Umweltforschung), Fraunhofer Institute for Molecular Biology and Applied Ecology IME, and Bayer AG will launch the collaborative research Project *DiMEP* (Digitale Muster Erkennung und Prädiktion). *DiMEP* aims to enhance the regulatory assessment of the potential for ED by utilizing molecular (transcriptomic) and phenotypic fingerprints. This involves employing the zebrafish embryo (ZFE) model as an animal-free alternative to juvenile/adult fish testing. The goal is to supplement the required Fish Early Life Stage (FELS, OECD 210) test with molecular pattern-based assessment to provide additional mechanistic information regarding the potential of a substance to cause endocrine effects.

The mission of *DiMEP* is to generate sublethal transcriptomic effect patterns (ecotoxicogenomic and phenotypic) in the zebrafish embryo for a sufficiently large set of known ED reference chemicals. Based on this high-dimensional data resource, computational tools and machine learning (ML) will be deployed to identify robust toxicogenomic patterns linked to known apical ED outcomes. This approach will enable a mechanistically informed identification of substances with the potential for ED in fish, likely reducing the need for additional fish-intensive studies.

### **1.02.P-Mo032 Advances Towards a Harmonized Environmental Safety Analytical Toolbox for Cosmetic Ingredients (ATEST)**

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The substantial use of laboratory animals for toxicity testing is highly cost-inefficient, and heavily against animal welfare considerations and the principles of the 3Rs (refine, reduce, replace). New Approach Methods or Non-Animal Methods (NAMs) offer a promising solution to avoid the need for *in vivo* animal tests. Although several NAMs have been proposed for environmental hazard assessments of chemicals, their maturity and stage of validation is limited or unclear. As chemicals, such as cosmetics and personal care products, can potentially produce diverse types of biological effects, there is a need to assemble a battery of NAMs with a broad coverage of endpoints for assessing the hazards of chemicals. The ATEST project is a thorough assessment of the current state of the science on available NAMs and their potential applications for cosmetic ingredients. The NAMs evaluation used an AI-based systematic review tool to identify relevant literature from public databases and the current state of the science of NAMs for environmental hazard assessments. A scoring system for assessing



the weight of evidence has been developed to rank NAMs for their maturity and regulatory applicability. Information related to the NAMs (e.g. measurement of certain ecotoxicological endpoints) were used as key words for querying public databases, through an established data mining workflow. The outcome of the ATEST project is a database describing the attributes of different NAMs identified for environmental safety including a ranking scheme to assess the reliability, relevance and regulatory acceptability of different NAMs. This will provide associations such as the International Collaboration on Cosmetics Safety (ICCS) with information on where future efforts are needed to improve emerging NAMs and promote the rapid transition from conventional to non-animal chemical hazard assessment.

#### **1.02.P-Mo033 Critical review of in vitro dosing methods for petroleum UVCB substances**

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Alternative approaches to traditional ecotoxicity tests, which include *in vitro* testing, are being promoted to support regulatory chemical assessments. *In vitro* test methods can be used in multiple contexts, from prioritization and screening to supporting chemical grouping and read-across. Eventually, it is anticipated that *in vitro* tests will be used in place of whole organism *in vivo* testing. An important challenge of most *in vitro* testing methods is how to establish, maintain and confirm defined test substance concentrations throughout the test. This is particularly challenging for petroleum UVCB substances (Unknown, Variable composition, Complex reaction products, or Biological origin) that typically contain a large number and variety of hydrophobic and (semi)volatile hydrocarbon constituents that are very prone to evaporative and sorptive losses.

The ability to deliver and maintain stable exposure of petroleum substances in *in vitro* test systems is challenged by several factors, including: high surface area to volume ratios of multi cell well plates, which increases the likelihood of sorption to plate walls; the inability to seal some test vessels (e.g., volatile constituents can escape open test vessels and may contaminate neighbouring cell wells); poor solubility of hydrophobic constituents in biological media and presence of lipids and proteins in biological media may differentially bind individual constituents. Here we present the first findings from a critical systematic review on the state of science of *in vitro* methods, their challenges, and their applicability in (eco)toxicological assessments of petroleum UVCBs. The outputs will be used to identify relevant *in vitro* tests and dosing methods and adaptations best suited for petroleum substances in future research and regulatory testing efforts.

#### **1.02.P-Mo035 Comparative in vitro characterization of biotransformation enzyme kinetics in different organs of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*)**

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Improving the applicability of *in vitro* methodologies to address chemical pollution in the aquatic environment requires specific knowledge about their metabolic competence, particularly for processes that drive chemical toxicokinetics, i.e. biotransformation. Currently, comparative studies regarding the presence, activity magnitudes, and inducibility of different biotransformation pathways are scarce for testing systems using fish. As a result, we here report a comparative characterization of organ-specific kinetic parameters for phase I and II biotransformation enzymes, under basal and induced conditions, using S9 sub-cellular fractions and four rainbow trout cell lines. Two of the most common biomarkers of biotransformation, CYP1A and glutathione S-transferase (GST), displayed their highest levels in liver S9 fractions and RTL-W1 liver cells. However, S9 fractions and cell lines derived from the intestine, gills, and brain also displayed these biotransformation pathways. In addition, while CYP3A4-like activity was only measured in liver and intestinal S9 fractions, all rainbow trout cell lines appeared to be equipped with this pathway. Basal CYP3A4-like activity was the highest in intestinal cells RTgutGC, but the inducibility of this pathway was higher in RTL-W1 cells. Similarly, while both RTL-W1 and RTgutGC cells appeared to be equipped with CYP2B-like activity, this was only measurable upon induction. In contrast, S9 fractions from the liver, intestine and gills displayed constitutive CYP2B-like activity. These differences among systems could be related to the complexity and potential differential functionality of biological processes at the *in vivo* level. Nonetheless, the biotransformation kinetic parameters obtained from our experimentation represent important evidence of the metabolic competence of fish *in vitro* methods. Based on our results, we suggest that both S9 fractions and cell lines are suitable testing alternatives for evaluations of organ-specific biotransformation of pollutants.

#### **1.02.P-Mo036 Characterization of rainbow trout hepatic 3D spheroids for next generation ecotoxicity testing**

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There is a growing need for animal alternatives using new approach methodologies (NAMs) for ecotoxicity testing of chemicals. There are currently few well-characterized *in vitro* (cell based) assays, based on both primary- and continuous cell cultures. Primary hepatocytes from rainbow trout (RT-HEP) have shown promise in acute toxicity assessments as they retain many of the native physiological functions of the liver. However, there are currently few cell-based alternative methods

available for long-term toxicity assessments. A novel three-dimensional (3D) spheroid model, derived from RT-HEP, has shown promise as it preserves many of its morphological, physiological and biochemical properties for weeks after isolation and formation. However, prior to application, the RT-3D hepatic cell culture system requires comprehensive characterization and validation. We therefore employed high-resolution multiphoton fluorescence microscopy (MFM) to access the morphology, area, diameter, sphericity, physiological structure, DNA/nucleus integrity and hypoxia. We further employed OMICS and various bioassays to comprehensively characterize the RT hepatic 3D spheroids. Model chemicals, representative of different toxic modes of action such as copper, 17  $\beta$ -ethinyl estradiol (EE2), pyrene, 3,4-dichloroaniline (DCA), and carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), were employed to assess the sensitivity and responsiveness of the RT-3D hepatic spheroids. Our assessment focused on analyzing the viability, metabolic activity, adenosine triphosphate (ATP) synthesis, vitellogenin (Vg) induction and oxidative stress response exhibited by these RT hepatic 3D spheroids when exposed to these specific model chemicals.

Initial findings suggest that the RT hepatic 3D spheroids serve as a reliable model for evaluating cytotoxicity, cell membrane integrity and oxidative stress. Additionally, the absence of hypoxia at the spheroid core was confirmed. Transcriptomics (RNA-seq) and untargeted metabolomics (mass spectrometry based) were also employed to better understand the molecular landscape of the 3D spheroids and their potential for assessing chemicals with distinct MoAs. Based on our ongoing characterization, the RT hepatic 3D spheroids model exhibits substantial potential as an ecotoxicity screening tool capable of assessing the risks associated with a diverse array of chemicals. This work was supported by the SPHERTOX Project (#324794) funded by the Research Council of Norway.

#### **1.02.P-Mo037 A review of *in silico* and *in vitro* methods for use in a risk assessment of a substance acting via oestrogen or androgen modalities**

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The testing of cosmetics on animals is banned in many countries, but manufacturers are responsible for demonstrating the safety of constituents for users and increasingly so for the environment. Of concern are those ingredients with endocrine disrupting properties. Therefore, the cosmetics industry requires an approach for conducting risk assessments for substances with potential endocrine activity which does not require *in vivo* animal testing, instead, demonstrating their safety using *In Vitro* to *In Vivo* Extrapolation (IVIVE) approaches. These approaches rely on the use of *in vitro* bioassays supported by predictive *in silico* data. Whilst there are many *in silico* models and *in vitro* methods which are used to predict the endocrine activity of chemicals, most have been optimised with a focus on classifying substances with endocrine activity rather than consideration of their biological relevance. A review of the available *in silico* and *in vitro* methods was therefore conducted to assess which were most suitable to support a human risk assessment for a substance acting via oestrogen or androgen modalities. The review considers several aspects including the relevance of the assay for humans (tissues and receptors) and for predicting effects in the whole organism, the sensitivity and specificity of the assay, applicability domain of the methods, as well as regulatory acceptability and commercial availability. We also considered whether these assays had already been used in IVIVE approaches and if so, whether the extrapolations were realistic. While this review has focused on humans, the findings are considered useful for informing on the prioritisation of New Approach Methodologies to use for risk assessments with non-target organisms, especially given the homology of oestrogen and androgen receptors in vertebrates.

#### **1.02.P-Mo038 Cytotoxic Disruption of Intracellular Parameters Induced by Thiacloprid in Mice Sertoli Cells *In vitro***

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Thiacloprid (2Z)-3-[(6-chloro-3-pyridinyl)methyl]-1,3-thiazolidin-2-ylidenecyanamide belongs to the neonicotinoid family, and it is mainly exploited in agriculture. At the same time, neonicotinoid pesticides are highly effective against some destructive crop pests, and their occurrence in aquatic ecosystems could represent a relevant risk. Although previous studies described the binding affinity of thiacloprid on nicotinic acetylcholine receptors (nAChRs) or  $\gamma$ -aminobutyric acid (GABA) receptors in insects, some information about the sensitivity of the reproductive system still resonates. Besides that, detailed knowledge about the potential mechanisms of effect and cytotoxic potential regarding other mammalian cells stays inconsistent. The primary aim of our *in vitro* study was to determine the potential impact of thiacloprid on mice Sertoli cells. TM4 cells were treated with experimental doses starting from 62,5  $\mu$ M to 500  $\mu$ M of thiacloprid for 48 hours. Screening of cytotoxic effects was evaluated based on the changes in mitochondrial activity, changes in cell membrane integrity, as well as through inhibition of lysosomal activity. The results from the mitochondrial activity assay (MTT) revealed that the whole panel of experimental concentrations inhibited this parameter. A significant ( $p < 0.0001$ ) reduction was recorded at 250  $\mu$ M, 300  $\mu$ M, and 500  $\mu$ M of thiacloprid. A similar tendency was observed in cell membrane integrity (CFDA-AM assay) evaluation. The higher concentrations than 125  $\mu$ M significantly ( $p < 0.001$ ) affected this parameter in exposed cells. A sensitivity of TM4 cells to thiacloprid exposure was confirmed via inhibition of lysosomal activity carried out by neutral red uptake. The panel of

experimental doses significantly ( $p < 0.0001$ ;  $p < 0.001$ ) decreased this parameter after 48 hours of exposure in vitro. Gained results of our pilot study suggest, that thiacloprid could affect intracellular parameters of Sertoli cells, and concerns about the negative impact on reproductive health are justified. A considerably more detailed and systematic research in thiacloprid toxicology is definitely required for a better understanding of risks associated with reproductive health.

Keywords: Sertoli cells, thiacloprid, mitochondrial activity, membrane integrity, lysosomes

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### 1.03.P Assessing Adverse Pollutant Effects on Host-Associated and Free-Living Microbiomes Using -Omics Approaches

#### 1.03.P-Mo039 Chemical Metabolomics – New Chemical Biology Tools to Explore Gut Microbiome Metabolism

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Introduction: Metabolites produced by the gut microbiome play a crucial and diverse role on host physiology, which are detectable in a wide range of biological samples including feces, plasma, urine, and cerebrospinal fluid. Microbiota dysbiosis has been associated with the development of diseases, however, the metabolic link has yet to be detected. The detailed and targeted analysis of these metabolites is important for the discovery of biomarkers, unknown bioactive molecules and toxic metabolites.

Materials and Methods: Mass spectrometric metabolomics is the method of choice for identification and quantification of these metabolites. Advanced methods at the interface of chemistry and biology coupled with metabolomics analysis are still limited. We have developed a unique and multifunctional chemoselective probe with synthetic  $^{13}\text{C}/^{12}\text{C}$  isotopically labelled analogs that allows for comparative and quantitative analysis of metabolites in human samples at low concentrations. We have termed this method **Quantitative Sensitive CHE**moselective **MetA**bolomics (*quant-SCHEMA*). Coupled to magnetic beads, this method allows the straightforward chemoselective extraction of metabolites from human samples. This isolation procedure of specific classes of metabolites from sample matrices led to significantly increased mass spectrometric sensitivity that facilitates the detection of metabolites at attomole quantities.

Results and Discussion: Our analysis of carbonyls, thiols, amines, and short-chain fatty acids (SCFAs) revealed previously unknown metabolites. We applied this methodology in dietary intervention study with 156 samples for nutritional biomarker discovery. We have successfully found four potential food biomarkers. The success of this large-scale application of this methodology results in further validation of this method and encourages the general use in metabolomics studies. We also utilized selective enzymatic treatment of metabolites in human samples for simplified identification of converted sulfated and glucuronidated metabolites to elucidate their chemical structure using mass spectrometry. Many of these identified phase II clearance products are linked to gut microbiota-human host co-metabolism.

Conclusions: We have successfully developed unique methods for the investigation of the metabolic interaction of host and microbes that identified more than 300 previously unknown dietary and microbial metabolites in human samples with unknown bioactivity.

#### 1.03.P-Mo040 Assessment of Taxonomic and Functional Changes in the Environmental Symbiotic and Free-living Microbiome Induced by Ag and Cu Metals

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Despite the importance of coastal ecosystems, their ecological well-being is threatened by the increasing release of anthropogenic sources of pollution. Metals are present in the environment as a result of natural or anthropogenic inputs. Silver is well known for its toxicity to organisms. Although its levels are generally low, the associated risk in coastal ecosystems is usually high due to sewage discharges. Copper is a widespread and well-known environmental contaminant. The development of pollutant exposure experiments in controlled microcosm systems makes it possible to simulate their effects in natural ecosystems. Due to their wide distribution and close contact with pollutants, microorganisms, both symbiotic and free-living in natural environments, could be good indicators of pollution. In this work, the changes in the microbial profile, both in sediments and in the digestive gland of the bivalve mollusc *Scrobicularia plana*, upon exposure to silver (Ag) or copper (Cu), or a mixture of both, at 1  $\mu\text{g/L}$  were characterised in an aquatic microcosm system. The phylum with the highest number of identifications in all samples was always Proteobacteria. The Desulfobactereaceae, Ectothiorhodospiraceae and Flavobacteriaceae families were highly abundant in all samples. After exposure to metals, there were a number of significant increases at the family level, highlighting Xanthomonadaceae in sediment samples after exposure to the Ag/Cu mixture with respect to individual exposure to Ag, and Chromatiaceae in digestive gland samples after individual exposure to Cu with respect to unexposed samples. These changes affected the functional potential of the microbiomes, as there was a significant increase in several functions, such as "aromatic degradation" and "hydrocarbon degradation", after exposure to the Ag/Cu

mixture compared to individual exposure to Ag in the sediment samples, and after individual exposure to Cu compared to the control in the digestive gland samples. Changes at the taxonomic and functional levels of the microbiome, symbiotic and free-living, have shown great potential as an environmental monitoring tool in coastal aquatic ecosystems.

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### **1.03.P-Mo041 Gut Microbiota of Zebra Mussels (*Dreissena polymorpha*) as a Holobiont Concept- Significantly Reliable Method for Aquatic Environment Monitoring?**

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France's Water bodies are constantly exposed to emerging pollutants from urban areas. It requires constant monitoring of contamination. Zebra mussel (ZM) is an invasive species which is spread in European waters extensively. They are filter feeders that filter a large amount of water as its food source. They process and remove a high number of microorganisms (MO). ZM accumulate compounds from the water column and is an excellent indicator of the surrounding environment. The filtration process allows ZMs to absorb naturally occurring mixtures and pollutants. ZM's gut microbiota (GM) contains a high number of MO and creates a specific symbiosis with the host. It is closely connected to the outside environment and represents aquatic conditions. It shows the importance of holobiont symbiosis and its need for detailed understanding. This study investigates the GM of ZM as an indicator of emerging contaminants such as human pathogens and antibiotics resistance bacteria (ARB). The inquiry is how GM is affected by variations of diet, pathogens and contaminants appearing in aquatic environments and the overall understanding of the gut microbiota of ZMs and its essence in the accumulation of increasing quantities of pathogens and ARB.

ZMs were collected in the field and let acclimated before following steps. After the acclimation period, the field investigation selected ZMs were transferred to appointed sites and put into specially created devices for collecting faeces. The rest of the ZMs were kept in laboratory conditions to further study their gastrointestinal microbiota. Further analysis will be performed by 16S rDNA sequencing and cultivation.

The experiment combines laboratory-controlled conditions and field experiments. Obtaining data from different feeding conditions, filtration rates and the overall effect of the laboratory and field environment and possible accumulation of pathogens and ARB from the field is critical for further study. Testing of water bodies can be more reliable with ZMs accumulation of contaminants with comparison to regular grab water samples depending on many factors.

This study emphasises the importance of the microbiota of filter feeders and their ability to accumulate compounds from the water.

### **1.03.P-Mo042 From dysbiosis to neuropathologies: toxic effects of glyphosate in zebrafish**

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Glyphosate, the most widely used herbicide worldwide, has long been assumed to have negligible effects on animals due to its selective inhibition of the shikimate pathway in plants. However, accumulating evidence suggests physiological impacts of glyphosate exposure in both humans and animal models, ranging from neurological effects to cancer. In addition to its recognized herbicidal properties, glyphosate also possesses a selective antimicrobial activity. In this study, we investigate the effects of environmentally relevant concentrations of glyphosate on the gut microbiota, neurotransmitter levels, and anxiety in zebrafish. Our findings demonstrate that glyphosate exposure leads to dysbiosis in the zebrafish gut, alterations in central and peripheral serotonin levels, increased dopamine levels in the brain, and notable changes in anxiety and social behavior. While the dysbiosis can be attributed to glyphosate's antimicrobial properties, the observed effects on neurotransmitter levels leading to the reported induction of oxidative stress in the brain indicate a novel and significant mode of action for glyphosate, namely the impairment of the microbiome-gut-axis. While further investigations are necessary to determine the relevance of this mechanism in humans, our findings shed light on the potential explanation for the contradictory reports on the safety of glyphosate for consumers.

### **1.03.P-Mo043 Examining the Interlinked Dynamics of Human Activities, Microbial Communities, and Greenhouse Gas Emissions in a highly impacted river system**

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Microbial communities play a vital role in freshwater ecosystems but are susceptible to adverse effects from human activities.

To enhance ecosystem management, it is imperative to comprehend their reactions. This study employed advanced sequencing techniques to explore the community composition and functional diversity of microbial communities in Wascana Creek, Southern Saskatchewan, Canada, a river system previously identified as highly impacted. Through targeted chemical analysis, suspect screening, and assessments of nutrients and greenhouse gases, the research aimed to pinpoint specific pollutants and characterize their presence in the creek. By combining these analytical techniques, we aimed to unravel the intricate interactions between anthropogenic activities and microbial communities, elucidating their responses and potential functional alterations. Conducted in 2022, the investigation exposed a significant human influence on Wascana Creek. Upstream of the City of Regina, agricultural practices led to increased phosphorus levels, causing shifts in microbial community composition and activity. Consequently, dissolved oxygen concentrations declined, resulting in fish mortality. Moreover, there was a noteworthy rise in methane emissions, a potent greenhouse gas. Downstream of the City of Regina wastewater treatment plant, the introduction of effluents led to substantial increases in anthropogenic pollutants and nitrogen, triggering significant changes in microbial community composition and functional diversity. These alterations aligned with heightened emissions of nitrous oxide (N<sub>2</sub>O), another potent greenhouse gas. These findings emphasize the direct influence of anthropogenic activities on microbial communities. Understanding the intricate interactions between anthropogenic activities, microbial communities, and ecosystem functioning is crucial. By examining microbial responses to anthropogenic stressors, valuable insights were obtained into the mechanisms underlying ecosystem disruption.

### **1.03.P-Mo044 Metal Pollution Affects the Early-life Gut Microbiota in Small Passerine Birds**

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Microbes play a significant role in terrestrial and aquatic ecosystems by acting as plant, animal and human pathogens, but many microbes have protective functions as well. Metals are well-known antimicrobial elements and metal pollution is a potential but little studied modifier of microbial environment in wild birds. Pollution-induced changes in gut microbiota may have serious effects on avian health and physiology. Particularly, early-life exposure to environmental pollutants may have great impact on the gut microbiota development and microbiota-dependent physiological functions, such as immune and stress responses. We studied the effects of metal pollution on early-life microbial environment of wild passerines by exploring bacterial diversity and community in nestlings' feces by 16S rRNA sequencing. We used three common model species, great tits (*Parus major*), blue tits (*Cyanistes caeruleus*) and pied flycatchers (*Ficedula hypoleuca*), which commonly breed at metal polluted areas in Finland. The field work was conducted near a copper-nickel smelter, an area with highest rates of metal pollution in Finland. Responses of microbiota to pollution, habitat, microclimate, taxonomy and dietary differences were measured and connected to ecological (brood size, growth, survival) measures of individual performance. The most common bacterial phyla were Firmicutes, Proteobacteria and Actinobacteria. Our preliminary results showed that the fecal microbial diversity (Shannon index) and richness (Chao1) did not differ between the study areas or between the species. However, the diversity decreased with increasing brood size in great tits and blue tits, while in pied flycatchers the diversity increased with nestling mass. Also, the fecal microbial communities differed between the species and study areas and several bacterial orders were associated with metal concentrations, nestling growth and fledging success. Moreover, several bacteria at family and genus level were differently abundant between the study areas and study species. The results suggest that metals may change the early-life bacterial communities in small passerine species living close to metal polluted areas.

### **1.03.P-Mo045 Distribution of metals and pesticides in spoonbills from natural areas of the southwest Spain. The impact on the microbial-produced metabolites and gut microbiota**

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The consequences of pollution due are widely recognized on a global, regional and local scale as well as their influence on ecosystems, especially in areas with abundant water, which acts as a carrier. The common spoonbill (*Platalea leucordia*) is a wetland-dwelling wading bird highly sensitive to pollution, making it a good bioindicator for assessing the ecological health of ecosystems. To this end, 70 feathers, 146 blood samples and 60 spoonbill eggs were analyzed in four selected areas during 2006 and 2011 (Southwest Spain): Odiel Marshes Biosphere Reserve (OM), marshland nature reserve of the Marismas de Isla Cristina (MIC), Doñana Natural Park and Natural Park of Cadiz Bay (CB). The presence of metals was evaluated by inductively coupled plasma-mass spectrometry (ICP-MS) while chlorinated organic compounds (PCBs and pesticides) were assessed by gas chromatography with an electron capture detector. High Fe content was observed in blood, along with lower levels of Mn, Ni and Se compared to the feather samples. Toxic metals such as Pb, Cd and Co were absent in blood and eggs. Regarding pesticides, DDT and, into a lesser extent,  $\beta$ -endosulfan were the most common, with the highest pesticide levels found in MIC. Similarly, this site point was the most contaminated area with PCBs, with the highest concentration found in the blood. A higher accumulation of metals was observed in feathers due to permanent exposure to contaminants, and the highest concentration of pesticides and PCBs was found in blood samples from MIC. Moreover, a new sampling campaign has been performed during 2023 collecting 4 samples of water, 4 samples of soils and 30 samples of faeces of spoonbills in CB. Other sampling points to increase the number of samples is ongoing. Pesticides and metals have been determined, while an untargeted metabolomic study has been performed in the faeces to determine the microbial-produced metabolites that could be potentially related with the presence of pollutants. Microbiota profiling was also determined by 16S amplicon sequencing in

Illumina platform. The metabolomic analysis was carried out by combining GC with mass spectrometry and ultra-high performance liquid chromatography coupled to quadrupole time of flight (UHPLC-QTOF). The potential gut metabolic and microbiota impairments caused by environmental pollution in the different sampling points as well as the potential associations with pollutants are under investigation.

### **1.03.P-Mo046 Microbial Changes in Treated Wastewater Irrigated Paddy Soils and the Impacts on Biogeochemical Cycles**

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Global freshwater withdrawals by the agricultural sector account for approximately 72%, placing it in strong competition with global cities. As a nutrient-rich resource, treated wastewater poses a viable option for meeting the high-water demand in agriculture, especially in water-intensive farming systems. However, conventional wastewater treatment processes do not eliminate all contaminants; thus, using treated wastewater (TWW) poses potential adverse risks for plants, agricultural soils, and the environment. We examined changes in microbial communities of paddy soils following one and three years of TWW irrigation. Our aim was to assess the microbial response to TWW and the consequences for biogeochemical cycles. After one year, pH, nutrients, and heavy metals significantly increased. However, most of the initially observed spikes had significantly recovered with three years of TWW irrigation, except for total carbon, nitrogen, and toxic elements including As, Pb, and Cr. Changes in soil physical and chemical properties were accompanied by differential abundance expression, with one year of TWW use showing more differentially abundant microbes than after three years. Notably, the differentially abundant microbes of the core taxa spanned across several phyla after one year (*Acidobacteria*, *Chloroflexi*, *Proteobacteria*, and *Nitrospirae*), compared to only *Chloroflexi* and *Nitrospirae* after three years. Interestingly, two rare taxa (*SBR1093* and *WS3*) significantly varied after three years. Moreover, alpha diversity indices were significantly low after three years. In addition to showing a lower abundance of microbes related to methane generation, TWW-irrigated fields had an overall reduction in methanogenesis and denitrification, highlighting the potential of TWW irrigation to alter microbial composition and subsequent processes crucial for organic matter mineralization and carbon sequestration.

### **1.03.P-Mo047 Beyond Nutrition: Breast milk Minerals Shape Infant Microbiota**

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Introduction: Breast milk (BM), a dynamic fluid influenced by the maternal exposome, raises intriguing questions about its impact on the infant's microbiota. This study explores the potential role of variations in BM minerals and heavy metal composition in shaping the infant gut microbiota.

Methods: BM samples from 98 healthy mothers from MAMI cohort at two stages, early and mature, during lactation were included. Clinical and anthropometrical data from mother-infants were available. Mineral profile was obtained by inductively coupled plasma atomic emission spectroscopy (ICP-AES) and microbial profile was obtained by high-throughput sequencing of the 16S rRNA gene in Illumina platform. Unsupervised clustering and statistical analyses, including sparse Partial Least Squares (sPLS) multi-omics integration, were applied to unravel mineral-based compositional patterns and explore mineral-microbial dynamics.

Results: Significant variations were observed in the mineral composition of early and mature BM. Cadmium, and lead increased, while molybdenum, copper, cobalt, antimony, and zinc decreased significantly. Infant fecal samples showed an increase in *Bifidobacterium* spp. and a decrease in *Klebsiella* spp. during first months. Analysis of variance revealed a significant interaction effect between k-means cluster and time on Shannon and Simpson diversity indices. Numerical differences in diversity were found to be lower in infants with early BM compared to those with mature BM in the mineral cluster 2. Mineral differences, particularly higher cadmium and lead concentrations and lower molybdenum and antimony levels in mature BM from cluster 2, were identified. sPLS analysis suggested a moderate positive influence of aluminium on *Bacteroides*, and arsenic on *Corynebacterium* in early BM samples. Negative loadings were identified on *Corynebacterium* for vanadium in early BM, and for copper and zinc in mature BM.

Conclusion: Shifts in mineral profile, including elevated molybdenum, cadmium, and lead, and reduced copper, cobalt, antimony, and zinc, were observed in mature BM compared to early milk samples. Significant interactions between clustered minerals and time on diversity suggest a potential influence of heavy metal levels on shaping the infant gut microbiota. Further investigation is required to understand the biological or clinical significance of mineral-microbiota relationships in the context of initial infant gut seeding.

### **1.03.P-Mo048 Potential associations of chiral thyroid hormones and microbiota in human milk**

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Iodine can only be obtained from the diet, and its requirements increase during pregnancy to fulfil altered maternal thyroid physiology and foetal development. There are previous studies related to the impact of iodine deficiency on the metabolome of human milk (HM). Thyroid hormones (TH) are the only compounds that contain iodine with biological activity and they are important for the infant's health. TH have chiral centers and their enantiomers present different biological and metabolic efficiency. Likewise, the levels of D-thyroxine (D-T4) do not influence the metabolic rate, but on the contrary, the levels of L-T4 do clearly influence it. However, D-T4 influences the levels of lipogenic enzymes in the liver, reduces cholesterol present in the plasmatic serum and decreases the secretion of thyrotropin (TSH). However, it is known that the efficiency of the L-T4 is much higher than that of its D form, but under our knowledge, chiral forms have not been previously reported in HM.

Herein, a novel analytical method was developed to separate 8 TH in HM using a column switching system mounted into an ultra-high performance liquid chromatography (UHPLC) that combines chiral and reversed stationary phases. TH were determined combining quadrupole time of flight (QTOF) tandem mass spectrometry, inductively coupled plasma triple quadrupole mass spectrometry (ICP-QQQ-MS) and ion mobility mass spectrometry (IMMS). Sample treatment involved hollow-fiber liquid-phase microextraction (HF-LPME) in three-phase mode. HF-LPME is a reliable method, simple and low cost that due to fiber disposability, avoids sample cross-contamination. The method allows determining 8 TH in HM at natural levels. The methodology has been applied to 134 HM samples. The concentration (ng/g) of TH followed the order D-T4 ( $96.78 \pm 164.00$ ) > L-T4 ( $71.23 \pm 112.65$ ) > 3,3',5'-triiodothyronine ( $65.22 \pm 112.54 = rT3$ ) > 3,3',5-triiodothyronine ( $43.85 \pm 68.43 = T3$ ) > 3,5-diiodothyronine ( $43.42 \pm 56.35 = T2$ ) > thyronine ( $19.94 \pm 34.41 = T0$ ) > 3,5-diiodotyrosine ( $9.86 \pm 21.86 = DIT$ ) > 3-iodotyrosine ( $9.53 \pm 26.27 = MIT$ ). Moreover, the microbiota profiling was obtained by 16S amplicon sequencing in Illumina platform and our results demonstrated a separation between mothers with iodine deficiency from control samples. The potential associations between clinical variables and the concentration of thyroid hormones with HM microbiota are under investigation.

### **1.03.PC Assessing Adverse Pollutant Effects on Host-Associated and Free-Living Microbiomes Using -Omics Approaches**

#### **1.04.A Behavioural Toxicology: Methodologies and Research Needs**

##### **1.04.A.T-01 Perceptions about the quality and utility of Behavioural (Eco)Toxicology to protect human and ecosystem health**

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A survey was conducted on the perceptions and role of behavioural ecotoxicology in the protection of the environment and human health. Those surveyed included environmental scientists working in the field of environmental toxicology and behavioural ecology and represented industry, government, NGOs and academia/research centres. Of the 166 respondents, 57% worked already with behavioural endpoints in ecotoxicology and/or ecology. The respondents agreed that contaminants can (97%) and are impacting wildlife (77%), and can (84%) and are impacting humans (62%). Those responding to questions about the impacts on humans resulted in more answers that were neutral or don't know. Overall respondents believed behavioural experiments to be repeatable (60%), reliable (61%) and relevant (84%), although those not studying behaviour were more cautious in their answers. Respondents were more likely to be neutral when asked whether behavioural endpoints are more sensitive (43%) but agreed (80%) that they provide important alternative information to standard endpoints. The largest overall response was to disagree with the statement that behavioural endpoints are currently used in risk assessment (42%) but the greatest proportion agreed they were essential (55%). The majority of respondents disagreed (63%) with the statement that we understood the risks of contaminants to wildlife and human health. However, 68% agreed that regulatory authorities should consider behavioural endpoints when making decisions over risk, with 78% of respondents agreeing this would likely come with increased costs for regulators and/or industry. Interestingly, 54% agreed and 28% were neutral that understanding the effects of contaminants on wildlife behaviour is critical to protecting human health. The largest proportion of respondents disagreed (35%) or were neutral (30%) with the statement that behavioural are easily linked to standardised apical endpoints and population-level effects. We discuss how this data could be used to further support our understanding and confidence in the effects of contaminants on human and wildlife behaviour and the mechanisms by which they can be incorporated into risk assessment and regulation.

##### **1.04.A.T-02 Impact Of Environmentally Relevant Concentrations Of Fluoxetine On Zebrafish Larvae: From Gene To Behavior**

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The selective serotonin reuptake inhibitor class of antidepressants (SSRIs) are usually prescribed for treatment of depressive states, acting at the level of the central nervous system, consequently affecting non-target organisms. Our work aimed to investigate the influence of environmentally relevant fluoxetine concentrations (1 to 1000 ng/L) on *Danio rerio* development, assessing both embryotoxicity and behavior, gene expression and neurotransmitter levels at larval stage. Exposure to fluoxetine during early development accelerated embryo hatching, reduced larval size and increased heart rate. Behavioral impairments (decreased startle response and increased larvae locomotor activity) were associated with effects on monoaminergic systems, detected through the downregulation of key genes (*vmat2*, *mao*, *tph1a* and *th2*) and with altered levels of neurochemicals belonging to the serotonergic and dopaminergic systems (increased levels of tryptophan and norepinephrine and decrease in 3,4 dihydroxyphenylacetic acid levels). Overall, the results highlight the sensitivity of early life stages of *Danio rerio* to low concentrations of fluoxetine, inducing effects that may compromise larval survival. The obtained data support the need to test low concentrations of SSRI in environmental risk assessment and the use of biomarkers at different levels of biological organization for a better understanding of modes of action.

#### **1.04.A.T-03 Impacts of pharmaceuticals on animal behaviour: a systematic map of evidence and open access database**

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Over the last decade, there has been a rapid resurgence of behaviour as a tool for assessing the impacts of emerging pollutants. Behaviour is proposed as a particularly sensitive indicator for contaminant-induced impacts on non-target species—especially compared to standard ecotoxicological endpoints; it can also act as a bridge, linking the immediate effects at the sub-organismal level to broader, ecologically relevant outcomes at the population level. Despite this, behaviour is rarely used in a regulatory context. Recently, there has been a concerted effort to integrate behavioural assessments into environmental regulation and risk assessment. To make the most well-informed decisions during this critical integration process, there is an urgent need for a systematic synthesis of currently available data. In essence, without knowing what we have done, how can we best guide practice moving forward? Accordingly, our team employed a large-scale systematic evidence map approach to identify, categorise, and visualise research detailing the effects of pharmaceuticals on the behaviour of aquatic animals. The primary objective was to create a large open-access database on the impacts of pollution on animal behaviour for regulators and researchers. In brief, we searched two electronic databases (Web of Science and Scopus) with an exhaustive search string structure using a PECO framework. The resulting articles were screened in two stages, title and abstract, followed by full-text screening. After removing duplicates, 5503 articles underwent title and abstract screening. In total, 1103 articles proceeded to full-text screening and metadata extraction. The metadata taken from the articles centred around four themes: (1) species information, (2) compound information, (3) behavioural measurements, and (4) quality criteria (based on the criteria for reporting and evaluating ecotoxicity data). In total, 774 individual articles were included in our final database, representing 1472 individual compound–species assessments. There were 405 compounds and 170 species identified in our database, representing over 1800 behavioural assays. During my presentation, I will use this new database to highlight current knowledge gaps, overrepresented study systems, and areas currently well-suited for meta-analysis approaches.

#### **1.04.A.T-04 EthoCRED: A Framework to Guide Reporting and Evaluation of the Relevance and Reliability of Behavioural Ecotoxicity Studies**

**Michael Grant Bertram<sup>1</sup>**, Marlene Ågerstrand<sup>2</sup> and Tomas Brodin<sup>1</sup>, (1)Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences (SLU), Sweden, (2)Stockholm University, Sweden

Behavioural analysis has been garnering significant attention as a broad indicator of sub-lethal toxicity, and has secured a place as an important sub-discipline in ecotoxicology. One of the most notable characteristics of behavioural research, compared to other established approaches in sub-lethal ecotoxicology (e.g. reproductive and developmental bioassays), is the wide range of study designs being used and the diversity of endpoints considered. At the same time, environmental hazard and risk assessment, which underpins regulatory decisions to protect the environment from potentially harmful chemicals, often recommends that ecotoxicological data be produced following accepted and validated test guidelines. These guidelines typically do not address behavioural changes, meaning that these, often sensitive, effects are not represented in hazard and risk assessments. In this presentation, we propose a new tool, the EthoCRED evaluation method, for assessing the relevance and reliability of behavioural ecotoxicity data, which considers the unique requirements and challenges encountered in this field. This method, and accompanying reporting recommendations, are designed to serve as an extension of the 'Criteria for



Reporting and Evaluating Ecotoxicity Data (CRED)' project. As such, EthoCRED can both accommodate the wide array of experimental design approaches seen in behavioural ecotoxicology, and is able to be readily implemented into regulatory frameworks in different jurisdictions to allow better integration of knowledge gained from behavioural testing into environmental protection. Furthermore, through our reporting recommendations, we aim to improve the reporting of behavioural studies in the peer-reviewed literature, and thereby increase their usefulness in chemicals regulation.

#### **1.04.P-Tu001 Behavioral consequences through telomere shortening in wildlife birds living in a chronic exposure of urban pollution**

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Urban pollution lead to oxidative stress. Telomeres (non-coding DNA at the end of the chromosomes) are particularly sensitive to oxidative damage, since ROS (Reactive Oxygen Species) can accelerate their normal shortening in length due to aging. Individual differences in telomere length are also associated with differences in behaviour: the shorter the telomeres, the more nervous the animals are. A possible explanation for this link could reside in the "telomere position effect" (TPE), or rather the silencing of the genes at the end of the chromosome by the spread of the telomeric heterochromatin. Therefore, the shorter a telomere becomes, the more the closer genes are expressed. TPE could alter the phenotype according to senescence, regulating the expression of specific genes involved in behaviour as the dopamine receptors. To test this hypothesis, we used a free living bird, the blue tit (*Cyanistes caeruleus*), as model. We collected a blood sample at days 8 and 15 after hatching from nestlings in artificial nest boxes along a gradient of air pollution, in order to assess their telomeric shortening during the juvenile phase. The oxidative status of some subjects was experimentally manipulated through Polyinosinic:polycytidylic acid (Poly I:C), a synthetic RNA that binds the Toll-like receptor 3 (TLR3) producing ROS. We have then studied the nestlings' behaviour in the bold-shy continuum in relation to their telomere shortening, through an open-field test we designed. Chicks raised from nests in the most polluted area expressed higher telomere loss compared to those in the less polluted zone. The treated individuals with Poly I:C showed accelerate telomere shortening compared to those who received a saline solution, potentially indicating a causal role of ROS in telomeric loss. Finally, the rate of individual's telomere shortening correlated with its agitation and bold personality. Our study provides support for an association between air pollution and telomeric loss, and for a relation between telomere shortening and personality traits. Our finding calls into question the role of telomere length in behavioural modulation, and we hypothesize that the mechanism of action may reside in the TPE. We hypothesize the adverse outcome pathways (AOP) for which chronic exposure to urban pollution may alter animal personality, through a different expression of genes that regulate behaviour by the telomere position effect, triggered by ROS-induced telomere shortening.

#### **1.04.P-Tu002 Exploring the Effects of Two Surface Coatings on Titanium Dioxide Nanoparticles in "Danio rerio" Embryos**

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The environmental safety of titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) is under increasing debate. Previous works showed that TiO<sub>2</sub> NPs with a primary size of 5 and 25 nm induce hepatocyte ultrastructural alterations, along with metabolic alterations, inflammation, and oxidative stress in fish. However, these effects depend on several variables, such as average particle size, route of exposure, and sensitivity/resistance of the model organism. Since the type of surface coating is also reported to affect the biological response of NPs, in the present work, we studied alterations induced by 45 nm TiO<sub>2</sub> NPs exposure with two different coatings [sodium citrate and N-(trimethoxysilylpropyl)ethylenediamine, triacetic acid (EDTA-silane)] using *Danio rerio* embryos. Three trials were performed to study (i) developmental effects (inc. hatching, length, and malformations), (ii) behaviour (inc. swimming distance and responses to light/dark transitions), and (iii) gene expression (alterations of genes involved in the regulation of oxidative stress - CAT and SOD, immune responses and inflammation - NFκB1 and PPARα, and lipid metabolism - ACOX1, CPT1α, PPARα, HADH, and ELOVL5). The concentrations tested ranged between 5 and 200 mg TiO<sub>2</sub> NPs/L.

Overall, the exposure to 45 nm TiO<sub>2</sub> NPs with the two tested coatings induced relatively low harmful effects on *D. rerio* embryos regarding early life development. However, gene expression alterations were observed to depend on the type of surface coatings. Genes related to lipid metabolism (HADH and ELOVL5), oxidative stress (SOD), and immune response (NFκB1) are differentially modulated by the type of coating, with EDTA-silane inducing more alterations (mostly down-regulation of genes) than sodium citrate. Besides, behavioural alterations (swimming distance and inactivity) were detected, suggesting more stress on organisms exposed to the EDTA-silane-coated than the citrate-coated TiO<sub>2</sub> NPs.

While waterborne exposure to 45 nm TiO<sub>2</sub> NPs may seem relatively less toxic to aquatic organisms when compared to other lower-sized NPs, some surface coatings may increase their reactivity and interaction with (bio)molecules, and the overall

toxicity to aquatic organisms. Given the immense range of parameters reported to affect the biological response to engineered nanomaterials exposure, general assumptions on nanoparticle safety/toxicity should be taken carefully.

#### **1.04.P-Tu012 Effects of Acute 6PPD-Quinone Exposure on Swimming Performance and Aerobic Metabolism in Juvenile Lake Trout (*Salvelinus namaycush*)**

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is a transformation product of the most widely used rubber tire antioxidant, 6PPD. Commonly found in road-way runoff, a variety of studies have reported this compound to cause acute lethality in several salmonid species at roughly  $\leq 1 \mu\text{g/L}$ , including lake trout (*Salvelinus namaycush*; 24 h  $\text{LC}_{50} = 0.51 \mu\text{g/L}$ ). However, there is currently a lack of research targeted at understanding sublethal toxicities of 6PPD-quinone exposure, particularly on swimming performance. Sensitive species show characteristic symptoms including gasping, spiraling, increased ventilation, loss of equilibrium and impaired swimming ability, suggesting a possible impact on cardiometabolic physiology. To evaluate potential effects of 6PPD-quinone on swimming performance and aerobic metabolism, juvenile lake trout were exposed to nominal aqueous concentrations of 0 or 0.5  $\mu\text{g/L}$  in a swim tunnel respirometer for 20 hours to assess temporal changes in standard metabolic rate (SMR) compared to unexposed controls. Following exposure, fish underwent a swim trial to determine critical swim speed ( $U_{\text{crit}}$ ), oxygen consumption ( $\text{MO}_2$ ), active metabolic rate (AMR), aerobic scope (AS) and energetic cost of transport (COT). After conducting the swim trial, fish were euthanized, and concentrations of muscle triglycerides and glycogen were determined. Preliminary data show that 6PPD-quinone exposure does not significantly impact oxygen consumption rates or energetic cost of transport during a critical swim test. In contrast, exposure appeared to cause a significant decrease in critical swim speed and active metabolic rate, providing evidence of altered metabolism. These results suggest that aqueous 6PPD-quinone exposure at environmentally relevant concentrations produces adverse effects that can diminish endurance and maximum swim speeds, which may affect survival of fish populations. This is the first study to analyze the toxicity of 6PPD-quinone on the swimming performance of fishes of commercial, cultural, and ecological importance and provides urgently needed information for environmental risk assessments of this emerging contaminant of concern.

#### **1.04.B Behavioural Toxicology: Methodologies and Research Needs**

##### **1.04.B.T-01 Environmental Concentrations of Tire Rubber-Derived 6PPD-Quinone Alter CNS Function in Zebrafish Larvae**

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-quinone) is a degradation product of 6PPD, an antioxidant widely used in rubber tires. 6PPD-quinone enters aquatic ecosystems through urban stormwater runoff and has been identified as the chemical behind the urban runoff mortality syndrome in coho salmon. However, the available data suggest that the acute effects of 6PPD-quinone are restricted to a few salmonid species and that the environmental levels of this chemical should be safe for most fish. In this study, larvae of a "tolerant" fish species, *Danio rerio*, were exposed to three environmental concentrations of 6PPD-quinone for only 24 h, and the effects on exploratory behavior, escape response, nonassociative learning (habituation), neurotransmitter profile, wake/sleep cycle and circadian rhythm were analyzed. Exposure to the two lowest concentrations of 6PPD-quinone resulted in altered exploratory behavior and habituation, an effect consistent with some of the observed changes in the neurotransmitter profile, including increased levels of acetylcholine, norepinephrine, epinephrine and serotonin. Moreover, exposure to the highest concentration tested altered the wake/sleep cycle and the expression of *per1a*, *per3* and *cry3a*, circadian clock genes involved in the negative feedback loop. The results of this study emphasize the need for further studies analyzing the effects of 6PPD-quinone in "tolerant" fish species.

##### **1.04.B.T-02 Comparative Assessment of the Behavioral Responses in Local Amphipod Species to Anthropogenic Stressors**

**Laura Johanna Soose<sup>1</sup>, Jörg Oehlmann<sup>1</sup>, Andreas Schiwy<sup>2</sup>, Henner Hollert<sup>2</sup> and Jonas Jourdan<sup>1</sup>,** (1)Aquatic Ecotoxicology, Goethe University Frankfurt, Germany, (2)Evolutionary ecology and environmental toxicology, Goethe University Frankfurt, Germany

Chemical pollution is increasing and one of the main drivers of biodiversity loss. Current methods for chemical risk assessment could be insufficient to detect subtle threats to biodiversity. Behavioral ecotoxicology offers the potential to detect adverse effects at environmentally relevant and sublethal concentration ranges. The lack of standardization is a major drawback for the use of behavioral endpoints in chemical regulation. To achieve standardization and incorporate environmental realism, it is important to establish stronger connections between ecological and ecotoxicological research. This can be accomplished, for example, by examining the interactions of local keystone species with realistic stressors. Anthropogenic environmental

stressors arise from various sources, such as diffuse inputs from agriculture that occur as event-related pulsed inputs following heavy rain events. Also point sources like wastewater treatment plants are relevant, causing continuous release of pollutants like pharmaceuticals and personal care products into aquatic systems. For both input pathways we developed behavioral assays using several local amphipod species with an optical behavior monitoring tool.

Our first test scenario represents pulsed pollutant exposure by tracking the behavioral response of the test organisms immediately after exposing them to stressors for 2 hours. First, we worked with *G. pulex*, a commonly used amphipod in ecotoxicology. We found that pesticides induced a more pronounced response than pharmaceutical compounds. We assumed that *G. pulex* might have some kind of familiarization effect due to its sampling location. Consequently, we expanded our species portfolio to the four most common local species. We found that species that are naïve to pollutant exposure reacted most strongly to pulsed exposure. In our second test scenario we focused on the effect of pre-exposure and the resulting behavioral response to light stimuli: We used a light-dark transition tests and were again able to identify differences between the local amphipods. With both behavioral assays, we can demonstrate the benefits of behavioral ecotoxicology and contribute to the debate on standardization.

#### **1.04.B.T-03 Sensitive Behavioural Endpoints and Generational Effects in *Daphnia magna* Upon Acute and Chronic Toxicity Testing**

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The crustacean *Daphnia magna* is a widely used model organism in ecotoxicological testing. So far, according to the OECD guidelines 202 and 211, immobility and reproduction are the main endpoints used for the determination of toxicity. Recent studies show, that sublethal concentrations of chemicals can result in behavioral changes in daphnids, which are not assessed in current guideline testing. Since these behavioral changes can have an effect on population-relevant traits of daphnids and therefore may affect the aquatic food chain, their implementation is of major importance.

Behavioural endpoints addressing the locomotor activity, phototaxis and chemotaxis of *D. magna* were tested in a 48-hour acute test as well as a 21-day chronic reproduction test (F<sub>0</sub> and F<sub>1</sub> generation) with the neurologically active insecticides imidacloprid, thiacloprid and fipronil. All have shown to cause alterations in swimming behaviour in sublethal concentration ranges and the behavioral parameters appear to be more sensitive than the classical endpoints immobilization and reproduction.

The data was evaluated under the aspect of the respective mode of action of the insecticides and according to the applicability of the behavioural endpoints allowing a predictability of substance effects. NOEC values for the behavioural parameters for all insecticides could be determined and an overview over the behavioural endpoints could be obtained regarding the sensitivity and reproducibility.

During the acute tests, the phototactic behaviour appeared to be the most sensitive parameter for imidacloprid and thiacloprid and the activity time for fipronil. During the chronic exposure tests, insights in behavioural changes of *D. magna* during development could be gained as well as the most sensitive parameters could be suggested. The phototactic behaviour seems to be the most sensitive parameter for imidacloprid at 48 hours and 7 days, the locomotor activity for thiacloprid and fipronil. The data of the F<sub>1</sub> generation born under exposure was compared to the data of the F<sub>0</sub> generation and the data from the acute behavioural tests. The F<sub>1</sub> generation has shown to be more sensitive under neonicotinoid exposure and did not show recovery during 48 hours without exposure. For the F<sub>1</sub> generation born under neonicotinoid exposure, the phototactic behaviour and locomotor activity were the most sensitive parameters, while no significant sensitivity could be shown for F<sub>1</sub> daphnids born under fipronil exposure.

#### **1.04.B.T-04 A method for assessing thermal preference and locomotion of a soil arthropod, *Folsomia candida* and its application in ecotoxicology**

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Conventional physiological endpoints like survival and reproduction are useful for environmental risk assessment (ERA) because they link directly to population dynamics, however, chemicals may also affect more subtle traits at low environmental concentrations, for example the behaviour of organisms. The behaviour of springtails is important for their persistence, ecological roles in decomposition and critical to survive extreme thermal conditions. However, standardised methods for assessing behavioural response of springtails to temperature or/and chemical exposure is lacking. This study aimed to investigate the thermal preference and locomotive response to temperature of springtails *Folsomia candida* under Cu contamination. We hypothesized that springtails would be behaviourally responsive to temperature and that Cu contamination can alter thermal preference and compromise mobility of springtails. Soils contaminated with three Cu levels (ca. 40, 400 and 1500 mg/kg dry soil) were collected from a former timber preservation industry at Hygum, Denmark. Age-synchronized springtails were exposed to the three exposure levels in Hygum soil for 3 weeks. In the thermal preference study, springtails were placed in the centre of rectangular arenas with a temperature gradient from 0 to 50 °C and the distribution were recorded after 5, 15, 30 and 60 mins. In the locomotion test, springtail was placed on a petri dish (diameter: 30 mm) at temperatures of 10, 15, 20, 25, 30, 33, 36, 40, 43 and 45 °C for 40 mins and the maximum velocity ( $V_{max}$ ), jumping intensity ( $J_{int}$ ), jumping

counts ( $J_{fre}$ ) and other locomotion parameters were calculated. Our results showed that the majority of springtails were able to adjust body temperature by moving along the temperature gradient to a preferred benign temperature, while few of them were trapped at thermal extremes. Cu exposure shifted the thermal preference of springtails and increased the risk of being trapped in the warm region of the arenas.  $V_{max}$  and  $J_{fre}$  also demonstrated unimodal relationship with temperature and was reduced by Cu exposure at high temperature. High  $V_{max}$  and  $J_{fre}$  matching with low occurrence at high temperature was possibly an escaping strategy for springtails. Increased  $J_{int}$  induced by extremely high temperature or Cu exposure suggested alterations in movement pattern. We conclude that assessment of toxic effects on thermal preference and locomotion is feasible and may be a valuable early-warning system for ERA.

#### **1.04.P-Tu003 Is Disrupted Reproductive Behaviour Population-Relevant?**

*Charles Hazlerigg<sup>1</sup>, Alice Tagliati<sup>1</sup>, Laurent L.-M. Lagadic<sup>2</sup>, Edward Salinas<sup>2</sup> and Thomas G Preuss<sup>2</sup>, (1)Enviresearch Ltd., United Kingdom, (2)Bayer AG - Crop Science Division, Germany*

Plant Protection Products and Biocides that are classified as EDCs will no longer be approved for use in the EU. Recently, there has been a growing interest in behavioural endpoints as EDCs have been shown to alter fish reproductive behaviours. Furthermore, environmental assessments are focussed on protection of wild populations, not individual organisms. Population models provide the potential to explore the population relevance of chemically-induced changes in individual behaviour. Here we present a study using a stickleback IBM to explore the population response to EDC-induced changes in reproductive behaviour; nesting and courtship behaviour.

After a 3-year spin-up period to stabilise the modelled stickleback population, hypothetical chemical effects were imposed disrupting the nesting and courtship behaviour of male stickleback. The behaviours were disrupted at four magnitudes: 10, 20, 50 and 90%. The effects were imposed continuously for 10 years. For each scenario, 70 simulations were performed. The population (abundance and biomass) response was evaluated across the entire 10 year effect period. The evaluation criteria used to determine an adverse population effect were taken from the recent EFSA Birds & Mammals guidance.

10 and 20% disruption in courtship behaviour did not lead to any adverse effects on the stickleback population. 50% disruption in courtship behaviour did lead to an observable reduction in abundance and biomass, whilst 90% disruption in courtship behaviour almost led to population extinction within the 10 years. Meanwhile, no population effects were observed following disruption of nesting behaviour at any of the four testing magnitudes of effect (data not shown). When comparing with effects on fecundity and sex ratio (male skew), the stickleback population was shown to be less sensitive to disruption of nesting and courtship behaviour.

Here we demonstrate how models can be used to assess the population relevance of disrupted behaviour observed in laboratory studies. Disruption of different behaviours have different population relevance. Results also show that other endpoints may be more relevant for population level assessment, making behavioural endpoints an indicator of endocrine activity rather than adversity in terms of the regulatory criteria.

#### **1.04.P-Tu004 A Multifactorial Environmental Stressors Approach: Chironomus riparius Exposed to Sulfoxaflor with Presence of Predator Cues and Increased Temperature**

*Sofie Bruun Rasmussen<sup>1</sup>, Thijs Bosker<sup>1</sup>, S. Henrik Barmantlo<sup>2</sup>, Olof Berglund<sup>3</sup> and Martina G. Vijver<sup>2</sup>, (1)Leiden University, Netherlands, (2)Institute of Environmental Sciences (CML), Leiden University, Netherlands, (3)Lund University, Sweden*

Growing evidence suggests that standard toxicity testing might underestimate the environmental risk of neurotoxic insecticides by not accounting for potential sublethal effects. Behavioural endpoints such as locomotion and mobility have been suggested as both sensitive and ecologically relevant alternatives to the standard tested endpoints to predict sublethal effects more accurately. Additionally, low levels of environmental realism in conventional toxicity testing raise concerns, as possible synergistic effects of a chemical and additional stressors are typically overlooked in standard risk assessment. Therefore, we aimed to investigate how concurrent exposure to environmental stressors and a nicotinic acetylcholine receptor (nAChR)-modulating insecticide ('sulfoxaflor') impact *Chironomus riparius* across a wide range of conventional and non-conventional responses.

To this end, a multifactorial experimental design encompassing three stressors, sulfoxaflor (at six different concentrations), predation risk (presence/absence of predatory cues), and temperature stress (20°C and 23°C), was used, yielding a total of 24 distinct treatment conditions. To assess potential additive effects, we applied the Independent Action (IA) model to predict the impact on eight endpoints, including standard endpoints (e.g., growth, survival, and emergence) and less conventional endpoints (e.g., swimming abilities and exploration behaviour). For the standard endpoints, observed effects were either lower than expected or well-predicted by the IA model. In contrast, we found synergistic effects of predation cues and temperature in combination with sulfoxaflor on adult size and larval exploration behaviour. Furthermore, the results indicated synergistic effects on larval swimming velocity between sulfoxaflor and increased temperature but not when combined with predation cues.

In conclusion, the results show that the total emergence of *C. riparius* is sensitive to the effects of sulfoxaflor, confirming their importance for standard ecotoxicity testing. However, we also found that conventional endpoints such as emergence, mortality,

and growth do not pick up on possible synergistic effects between the neurotoxic insecticide and important environmental stressors: predation cues and increased temperature. Awareness of this disparity will be critical for future studies and to improve risk assessment.

#### **1.04.P-Tu005 Effects of Chemical Stresses on the Freshwater Mussel *Dreissena polymorpha*'s Crawling Behaviour**

*Nicolas Berthelot<sup>1</sup>, Melissa Palos Ladeiro Dr.<sup>1</sup>, Mathilde Orquevaux<sup>2</sup> and Alain Geffard Pr<sup>1</sup>, (1)UMR-I 02 Sebio, University of Reims Champagne-Ardenne (URCA), France, (2)Direction de l'Eau et de l'Assainissement, Communauté urbaine du Grand Reims, France*

Bivalves are relevant organisms in which it can be measured biological responses (biomarkers) for water toxicity assessment. In this context the freshwater zebra mussel, *Dreissena polymorpha*, is a widespread sentinel species. Behaviors are known as biomarkers offering early responses to stresses. In bivalves there are many methods based on valvometry measurement (valves closures). But in *D. polymorpha* the other behaviors, as crawling, are largely under-researched. So, the present work aims to answer the question: can the locomotion be modulated by chemical stress in zebra mussels? Experiments were conducted in laboratory on young (10-15 mm) mussels. It consists of seven separately exposures (7 days) to copper, lead, triclosan, metformin, fluoxetine, and ofloxacin at 0; 1; 10; 100 and 1.000 µg/L, and to chlorpyrifos at 0; 0,1; 1; 10 and 100 µg/L. After the exposure step the crawling of mussels for each chemical condition is recording by video tracking. Significantly lower distances were registered in organisms exposed to 100 and 1.000 µg/L of copper with a mean individual distance of 19.22 mm for 0 µg/L but only 35 mm and 0.88 mm for 100 and 1.000 µg/L respectively. The mobility rate (number of mussels which have moved) is also significantly linked to the concentration, with 13 unmoving mussels for 0 µg/L and 25 unmoving mussels for 1000 µg/L. These results could be explain by the fact that copper is a biocide and it is attested as decreasing mobility in other bivalves. For fluoxetine exposure, mean individual distance is 12.63 mm for 0 µg/L exposed mussels while it is significantly higher (26.38 mm and 33.60 mm respectively) for 100 and 1.000 µg/L. The mobility rate did not depend on fluoxetine concentration. The increased crawling distances could be result from a stimulant effect of the fluoxetine regarding the mode of action of this pharmaceutical which is a selective inhibitor of the serotonin reuptake. To complete the discussion about present results with copper and fluoxetine other parameters as speed or delay before crawling can be used for describe the mobility. This work is in our knowledge the first evidences of chemical exposures effects on the locomotion in *D. polymorpha*. These present results show that *D. polymorpha* locomotion can be modulate under a chemical stress by increasing as well as decreasing traveled distances, showing a promising potential for crawling as futur tool in biomonitoring.

#### **1.04.P Behavioural Toxicology: Methodologies and Research Needs**

##### **1.04.P-Tu001 Behavioral consequences through telomere shortening in wildlife birds living in a chronic exposure of urban pollution**

*Matteo Schiavinato<sup>1</sup>, Shivani Ronanki<sup>2</sup>, Ignacio Miro Estruch<sup>3</sup>, David Spurgeon<sup>4</sup> and Nico van den Brink<sup>1</sup>, (1)Wageningen University & Research (WUR), Netherlands, (2)Department of Toxicology, Wageningen University & Research (WUR), Netherlands, (3)Toxicology, Wageningen University & Research (WUR), Netherlands, (4)UK Centre for Ecology & Hydrology (UKCEH), United Kingdom*

Urban pollution lead to oxidative stress. Telomeres (non-coding DNA at the end of the chromosomes) are particularly sensitive to oxidative damage, since ROS (Reactive Oxygen Species) can accelerate their normal shortening in length due to aging. Individual differences in telomere length are also associated with differences in behaviour: the shorter the telomeres, the more nervous the animals are. A possible explanation for this link could reside in the "telomere position effect" (TPE), or rather the silencing of the genes at the end of the chromosome by the spread of the telomeric heterochromatin. Therefore, the shorter a telomere becomes, the more the closer genes are expressed. TPE could alter the phenotype according to senescence, regulating the expression of specific genes involved in behaviour as the dopamine receptors. To test this hypothesis, we used a free living bird, the blue tit (*Cyanistes caeruleus*), as model. We collected a blood sample at days 8 and 15 after hatching from nestlings in artificial nest boxes along a gradient of air pollution, in order to assess their telomeric shortening during the juvenile phase. The oxidative status of some subjects was experimentally manipulated through Polyinosinic:polycytidylic acid (Poly I:C), a synthetic RNA that binds the Toll-like receptor 3 (TLR3) producing ROS. We have then studied the nestlings' behaviour in the bold-shy continuum in relation to their telomere shortening, through an open-field test we designed. Chicks raised from nests in the most polluted area expressed higher telomere loss compared to those in the less polluted zone. The treated individuals with Poly I:C showed accelerate telomere shortening compared to those who received a saline solution, potentially indicating a causal role of ROS in telomeric loss. Finally, the rate of individual's telomere shortening correlated with its agitation and bold personality. Our study provides support for an association between air pollution and telomeric loss, and for a relation between telomere shortening and personality traits. Our finding calls into question the role of telomere length in behavioural modulation, and we hypothesize that the mechanism of action may reside in the TPE. We hypothesize the adverse outcome pathways (AOP) for which chronic exposure to urban pollution may alter animal personality, through a different expression of genes that regulate behaviour by the telomere position effect, triggered by ROS-induced telomere shortening.

##### **1.04.P-Tu002 Exploring the Effects of Two Surface Coatings on Titanium Dioxide Nanoparticles in "Danio rerio" Embryos**

*Mário Araújo<sup>1</sup>, Elza Fonseca<sup>1</sup>, Elizabeth Bigorra-Ferré<sup>1</sup>, Carla Ferreira Melo<sup>2</sup>, Sergio Fernández-Boo<sup>1</sup>, Laura Rodriguez-Lorenzo<sup>3</sup>, Miguel Oliveira<sup>4</sup>, Vitor Vasconcelos<sup>1,5</sup>, Begõña Espind<sup>6</sup> and Alexandre Campos<sup>1</sup>, (1)University of Porto, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Portugal, (2)University of Aveiro, Portugal,*

(3)Water Quality Group, International Iberian Nanotechnology Laboratory (INL), Portugal, (4)University of Aveiro & Centre for Environmental and Marine Studies (CESAM), Portugal, (5)Department of Biology, Faculty of Sciences, University of Porto, Portugal, (6)International Iberian Nanotechnology Laboratory, Portugal

The environmental safety of titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) is under increasing debate. Previous works showed that TiO<sub>2</sub> NPs with a primary size of 5 and 25 nm induce hepatocyte ultrastructural alterations, along with metabolic alterations, inflammation, and oxidative stress in fish. However, these effects depend on several variables, such as average particle size, route of exposure, and sensitivity/resistance of the model organism. Since the type of surface coating is also reported to affect the biological response of NPs, in the present work, we studied alterations induced by 45 nm TiO<sub>2</sub> NPs exposure with two different coatings [sodium citrate and N-(trimethoxysilylpropyl)ethylenediamine, triacetic acid (EDTA-silane)] using *Danio rerio* embryos. Three trials were performed to study (i) developmental effects (inc. hatching, length, and malformations), (ii) behaviour (inc. swimming distance and responses to light/dark transitions), and (iii) gene expression (alterations of genes involved in the regulation of oxidative stress - CAT and SOD, immune responses and inflammation - NFkB1 and PPARab, and lipid metabolism - ACOX1, CPT1ab, PPARab, HADH, and ELOVL5). The concentrations tested ranged between 5 and 200 mg TiO<sub>2</sub> NPs/L.

Overall, the exposure to 45 nm TiO<sub>2</sub> NPs with the two tested coatings induced relatively low harmful effects on *D. rerio* embryos regarding early life development. However, gene expression alterations were observed to depend on the type of surface coatings. Genes related to lipid metabolism (HADH and ELOVL5), oxidative stress (SOD), and immune response (NFkB1) are differentially modulated by the type of coating, with EDTA-silane inducing more alterations (mostly down-regulation of genes) than sodium citrate. Besides, behavioural alterations (swimming distance and inactivity) were detected, suggesting more stress on organisms exposed to the EDTA-silane-coated than the citrate-coated TiO<sub>2</sub> NPs.

While waterborne exposure to 45 nm TiO<sub>2</sub> NPs may seem relatively less toxic to aquatic organisms when compared to other lower-sized NPs, some surface coatings may increase their reactivity and interaction with (bio)molecules, and the overall toxicity to aquatic organisms. Given the immense range of parameters reported to affect the biological response to engineered nanomaterials exposure, general assumptions on nanoparticle safety/toxicity should be taken carefully.

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To this end, a multifactorial experimental design encompassing three stressors, sulfoxaflor (at six different concentrations), predation risk (presence/absence of predatory cues), and temperature stress (20°C and 23°C), was used, yielding a total of 24 distinct treatment conditions. To assess potential additive effects, we applied the Independent Action (IA) model to predict the impact on eight endpoints, including standard endpoints (e.g., growth, survival, and emergence) and less conventional endpoints (e.g., swimming abilities and exploration behaviour). For the standard endpoints, observed effects were either lower than expected or well-predicted by the IA model. In contrast, we found synergistic effects of predation cues and temperature in combination with sulfoxaflor on adult size and larval exploration behaviour. Furthermore, the results indicated synergistic effects on larval swimming velocity between sulfoxaflor and increased temperature but not when combined with predation cues.

In conclusion, the results show that the total emergence of *C. riparius* is sensitive to the effects of sulfoxaflor, confirming their importance for standard ecotoxicity testing. However, we also found that conventional endpoints such as emergence, mortality, and growth do not pick up on possible synergistic effects between the neurotoxic insecticide and important environmental stressors: predation cues and increased temperature. Awareness of this disparity will be critical for future studies and to improve risk assessment.

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*Nicolas Berthelot<sup>1</sup>, Melissa Palos Ladeiro Dr.<sup>1</sup>, Mathilde Orquevaux<sup>2</sup> and Alain Geffard Pr<sup>1</sup>, (1)UMR-I 02 Sebio, University of Reims Champagne-Ardenne (URCA), France, (2)Direction de l'Eau et de l'Assainissement, Communauté urbaine du Grand Reims, France*

Bivalves are relevant organisms in which it can be measured biological responses (biomarkers) for water toxicity assessment. In this context the freshwater zebra mussel, *Dreissena polymorpha*, is a widespread sentinel species. Behaviors are known as biomarkers offering early responses to stresses. In bivalves there are many methods based on valvometry measurement (valves closures). But in *D. polymorpha* the other behaviors, as crawling, are largely under-researched. So, the present work aims to answer the question: can the locomotion be modulated by chemical stress in zebra mussels? Experiments was conducted in laboratory on young (10-15 mm) mussels. It consists of seven separately exposures (7 days) to copper, lead, triclosan, metformin, fluoxetine, and ofloxacin at 0; 1; 10; 100 and 1.000 µg/L, and to chlorpyrifos at 0; 0,1; 1; 10 and 100 µg/L. After the exposure step the crawling of mussels for each chemical condition is recording by video tracking. Significantly lower distances were registered in organisms exposed to 100 and 1.000 µg/L of copper with a mean individual distance of 19.22 mm for 0 µg/L but only 35 mm and 0.88 mm for 100 and 1.000 µg/L respectively. The mobility rate (number of mussels which have moved) is also significantly linked to the concentration, with 13 unmoving mussels for 0 µg/L and 25 unmoving mussels for 1000 µg/L. These results could be explain by the fact that copper is a biocide and it is attested as decreasing mobility in other bivalves. For fluoxetine exposure, mean individual distance is 12.63 mm for 0 µg/L exposed mussels while it is significantly higher (26.38 mm and 33.60 mm respectively) for 100 and 1.000 µg/L. The mobility rate did not depend on fluoxetine concentration. The increased crawling distances could be result from a stimulant effect of the fluoxetine regarding the mode of action of this pharmaceutical which is a selective inhibitor of the serotonin reuptake. To complete the discussion about present results with copper and fluoxetine other parameters as speed or delay before crawling can be used for describe the mobility. This work is in our knowledge the first evidences of chemical exposures effects on the locomotion in *D. polymorpha*. These present results show that *D. polymorpha* locomotion can be modulate under a chemical stress by increasing as well as decreasing traveled distances, showing a promising potential for crawling as futur tool in biomonitoring.

**1.04.P-Tu006 Chronic Exposure of Environmentally Relevant Concentration of Tire Derived Chemical Causes Anxiety-like Behavior and Memory Deficits in Adult Zebrafish**

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The presence of 2-((4-Methylpentan-2-yl)amino)-5-(phenylamino)cyclohexa-2,5-diene-1,4-dione (6PPDQ), an oxidation product of the tire antioxidant N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), has been known as the causative substance for a syndrome of mass mortality in Coho salmon before spawning in the northwestern United States [1]. Furthermore, tire-derived chemical have been found to exert a wide range of toxic effects on various species of fish. And these studies reported abnormal behavior such as ventilation failure, gasping, spiraling, and loss of equilibrium [2]. We hypothesized that these fish's behaviors were induced due to neurotoxicity, and we assess changes in neurobehaviors in adult zebrafish after chronic exposure to them at environmentally relevant concentrations. And neurosteroid analysis was conducted to unravel the underlying neurotoxicity mechanism.

Overall, we demonstrated the commonality in potential neurotoxicity of 6PPD and 6PPDQ as confirmed by neurobehavior assessment and neurosteroid analysis. Even taking into account the difference exposure methods and durations, both 6PPD and 6PPDQ caused anxiety-like behaviors in NTT assay and did not memory and learning deficits in T-maze test. Also, it is highlighted that profiles of neurosteroids affected by both 6PPD and 6PPDQ were similar, showing that Allopregnanolone and

its precursor Progesterone, Estradiol, Testosterone associated with GABA<sub>A</sub> and NMDA receptor dysfunction were reduced. The alteration of these neurosteroids well explain the anxiety-like neurobehavioral inducement and also suggests the potential for neurotoxicity associated with memory, excitatory, etc. although memory deficits were not observed in neurobehavioral phenotypic observations. Our findings provide the insight to more deeply explore the similarity of neurotoxicity mechanism of 6PPD and 6PPDQ, and further related studies should be conducted to better understand the toxicity of both substances.

1] Tian, Z., Zhao, H., Peter, K. T., Gonzalez, M., Wetzel, J., Wu, C., ... & Kolodziej, E. P. 2021. A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science*. 371(6525). 185-189.

[2] Brinkmann, M., Montgomery, D., Selinger, S., Miller, J. G., Stock, E., Alcaraz, A. J., ... & Wiseman, S. 2022. Acute toxicity of the tire rubber-derived chemical 6PPD-quinone to four fishes of commercial, cultural, and ecological importance. *Environmental Science & Technology Letters*. 9(4). 333-338.

#### **1.04.P-Tu007 From behaviour to gene transcription. Unraveling the molecular mechanisms that regulate diel vertical migration in the zooplanktonic *Daphnia magna***

*María Paula Carrillo Losada*<sup>1</sup>, *Benjamin Pina*<sup>1</sup>, *Maria Vila-Costa*<sup>2</sup> and *Carlos Barata Dr*<sup>2</sup>, (1)*Environmental Chemistry, Institute for Environmental Assessment and Water Research, Spanish Research Council (IDAEA-CSIC), Spain*, (2)*Institute for Environmental Assessment and Water Research, Spanish Research Council (IDAEA-CSIC), Spain*

Animal behaviour is closely related with individual fitness allowing animals to choose suitable mates or avoid predation. The central nervous system (CNS) regulates many aspects if not all of animal behaviour responses, therefore behavioural responses can be special sensitive to compounds with a neurodevelopmental or neuro-functional mode of action. Phototactic behavioural changes against fish in the freshwater crustacean *Daphnia magna* have been the subject of many ecological and more recently genomic investigations. The aim of this study was to identify the molecular pathways that modulate the phototactic behaviour to fish kairomones. We used two clones showing two contrasting responses. A positive phototactic clone (P<sub>132,85</sub>) that shows a marked negative phototactism after exposure to fish kairomones, to the muscarinic acetylcholine receptor scopolamine, and additive effects when exposed to both treatments; and a negative phototactic clone F that shows the opposite behaviour was . Fish conditioned water was obtained with juveniles of *Leuciscus idus*. Adults of both clones were exposed to three treatments plus their respective unexposed control: fish kairomones and scopolamine alone and its mixture. Whole transcriptomic illumina analyses indicated a greater number of de-regulated genes of the fish kairomone sensitive clone P<sub>132,85</sub> (1650) than the F one (1138), which were grouped in four clusters (two per clone). Two of the clusters (A) grouped genes down-regulated by the tested treatments, whereas in clusters (B) genes were up-regulated. In both clones gene transcription there was also additivity in animals exposed to both scopolamine and fish kairomone treatments. From the 35 enriched functional KEGG pathways 11 were shared by all clusters, whereas 14 and 5 were unique for clones P<sub>132,85</sub> and F, respectively. Over 50% of the shared and clonal specific metabolic routes were related with neurological pathways and regulation of cell proliferation/differentiation, whereas specific routes of the most responsible clone P<sub>132,85</sub> included the Wnt signaling pathway. Our results indicated that fish kairomone de-regulate not only neurological signalling routes but also cell differentiation/proliferation ones that are linked with the observed behavioural responses and the reported morphogenetic effects.

#### **1.04.P-Tu008 Impacts of a Psychoactive Pollutant on Interactions Between Native and Invasive Freshwater Fish** *Raiko Rafeeq*<sup>1</sup>, *Minna Saaristo*<sup>2</sup> and *Bob Wong*<sup>3</sup>, (1)*School of Biological Sciences, Monash University, Melbourne, Australia*, (2)*Environment Protection Authority Victoria, Australia*, (3)*School of Biological Sciences, Monash University, Australia*

As the need for medications continues to rise worldwide, pharmaceuticals are being increasingly detected in the environment. In particular, the antidepressant venlafaxine has become one of the most pervasive pharmaceutical pollutants in aquatic systems. The receptors targeted by venlafaxine are conserved across most animal groups, and venlafaxine can therefore affect ecologically important traits in non-target organisms. Disruptions to behavioural traits, such as boldness and aggression, can lead to changes in both intra- and interspecific interactions, causing further disturbances to vital ecological processes, including competition and predation. These interactions also play a key role in mediating invasion success, but research on the impacts of chemical pollution on native-invasive species interactions remains scarce. As such, our study aimed to investigate whether exposure to environmentally realistic concentrations of venlafaxine affected interspecific behavioural interactions between the southern pygmy perch (*Nannoperca australis*), a small freshwater fish native to Australia, and the invasive eastern mosquitofish (*Gambusia holbrooki*). Fish were exposed to either an unexposed freshwater control (i.e., no venlafaxine), low-venlafaxine exposure (0.5 µg/L), or high-venlafaxine exposure (1 µg/L) for 28 days. Following the exposure period, individuals were matched with a heterospecific from the same exposure treatment and both fish completed a behavioural trial to test aggression and foraging behaviours in the presence of a competitor. Preliminary results show that whilst there were no significant effects of venlafaxine treatment on aggressive behaviours in either species, mosquitofish in the high treatment were faster to first consume a prey item in comparison to the control treatment. This effect was not observed in southern pygmy perch, demonstrating a species-dependent effect of venlafaxine. A possible consequence of this effect could be that non-natives may outcompete native species, potentially leading to cascading effects on community structure and function. These findings highlight the need to better understand the complex interactions between different forms of human-induced environmental change.

#### **1.04.P-Tu009 Cardiac and Neurobehavioral Impairments in Three Phylogenetically Distant Aquatic Model Organisms Exposed to Environmentally Relevant Concentrations of Boscalid**



**Juliette Bedrossiantz<sup>1</sup>, Júlia Goyenechea<sup>2</sup>, Eva Prats<sup>3</sup>, Cristian Gómez-Canela<sup>4</sup>, Carlos Barata Dr<sup>5</sup>, Jérôme Cachot<sup>6</sup> and Demetrio Raldua<sup>1</sup>,** (1)Environmental Toxicology, Institute for Environmental Assessment and Water Research, Spanish Research Council (IDAEA-CSIC), Spain, (2)IQS School of Engineering, Ramon Llull University, (3)CID-CSIC, Spain, (4)School of Engineering, Institut Químic de Sarrià-Universitat Ramon Llull, Spain, (5)Institute for Environmental Assessment and Water Research, Spanish Research Council (IDAEA-CSIC), Spain, (6)EPOC CNRS, University of Bordeaux, France

Boscalid (2-Chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide), a pyridine carboxamide fungicide, is an inhibitor of the complex II of the respiration chain in fungal mitochondria. As boscalid is only moderately toxic for aquatic organisms ( $LC_{50} > 1$  -10 mg/L), current environmental levels of this compound in aquatic ecosystems, in the range of ng/L- $\mu$ g/L, are considered safe for aquatic organisms. In this study, we have exposed zebrafish, Japanese medaka and *Daphnia magna* to a range of concentrations of boscalid (1-1000  $\mu$ g/L) for 24-h, and the effects on heart rate (HR), basal locomotor activity (BLA), visual motor response (VMR), startle response (SR), and habituation (HB) to a series of vibrational or light stimuli have been evaluated. Moreover, changes in the profile of the main neurotransmitters have been determined. Boscalid altered HR in a concentration-dependent manner, leading to a positive or negative chronotropic effect in fish and *D. magna*, respectively. While boscalid decreased BLA and increased VMR in *Daphnia*, these behaviors were not altered in fish. For SR and HB, the response was more species- and concentration-specific, with *Daphnia* exhibiting the highest effects. At the neurotransmission level, boscalid exposure decreased the levels of L-aspartic acid in fish larvae and increased the levels of dopaminergic metabolites in *Daphnia magna*. Our study demonstrates that exposure to environmental levels of boscalid alters cardiac rate, impairs ecologically relevant behaviors, and leads to changes in different neurotransmitter systems in phylogenetically distinct vertebrate and invertebrate models. Thus, the results presented emphasize the need to review the current regulation of this fungicide.

#### **1.04.P-Tu010 The C.elegans as a Reliable Tool in Neuro-Behavioural Toxicology: Case study of Diesel Exhaust Particles**

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Exposure to particulate matter is increasingly associated with a heightened risk of neurodegenerative diseases, particularly Alzheimer's disease. This study investigates the behavioural and neurotoxic effects of diesel exhaust particles (DEP) on glutamatergic and dopaminergic neurons in the model organism *Caenorhabditis elegans* (*C. elegans*). Locomotion behaviours were assessed in two modes: swimming in a liquid medium using the Wmicrotracker system and crawling path and velocity analysis using the Image J system with captured video on NGM agar plates. A significant reduction in average speed ( $p < 0.001$ ), altered crawling paths, and decreased swimming activities ( $p < 0.01$ ) were observed under mid (0.167  $\mu$ g/cm<sup>2</sup>) to higher (1.67  $\mu$ g/cm<sup>2</sup>) concentrations. Evaluation of neurodegeneration markers in glutamatergic (DA1240) transgenic worms revealed notable degeneration in glutamatergic neurons at both concentrations. At 0.167  $\mu$ g/cm<sup>2</sup>, approximately 30% exhibited moderate degeneration, with 20% showing advanced degeneration. At 1.67  $\mu$ g/cm<sup>2</sup>, around 28% displayed moderate degeneration, and 24% demonstrated advanced degeneration ( $p < 0.0001$ ). Dopaminergic neurons (BZ555 transgenic strain) exhibited structural deformities without significant degeneration. In summary, DEP exposure induces distinctive neurodegenerative effects in *C. elegans*, with glutamatergic neurons being more vulnerable, suggesting a potential link between locomotion defects and glutamatergic neurodegeneration.

Keywords: Diesel exhaust particles (DEP), *C. elegans*, behaviour, Glutamatergic neurons, and Dopaminergic neurons, Neurodegeneration

#### **1.04.P-Tu011 Neurotoxicity evaluation of $\beta$ -citronellol, a major component of air freshener, in adult zebrafish**

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$\beta$ -citronellol is a substance incorporated into perfumes and various household chemical products for fragrance enhancement.  $\beta$ -citronellol has been known to have positive effects at low concentrations, such as suppressing the activity of nerve cells related to pain signals, but its effects at high concentrations are not studied. Therefore, in this study, changes in neurobehaviors were evaluated in adult zebrafish after exposure to  $\beta$ -citronellol in adult zebrafish, and the alteration in endogenous concentration of a total of neurochemicals and neurosteroids were measured in the brain using LC-MS/MS to explore the neurotoxicity mechanism. Male zebrafish were exposed to  $\beta$ -citronellol for 14 days at concentrations of 0, 0.04, 0.2, and 1.0 mg/L. Considering the stability of  $\beta$ -citronellol and the fragrance exposure scenarios, we conducted exposure for 4 hours daily. The test solution was changed every day to maintain the concentration of  $\beta$ -citronellol. The exposure conditions were determined based on instrumental analysis result that measured changes in the concentration of  $\beta$ -citronellol in the exposure tank. After exposure, three behavioral assays (i.e., novel tank test (NTT), photomotor response test (PMR) and T-maze test) were performed immediately to investigate neurobehavioral changes in response to  $\beta$ -citronellol. Neurotransmitters and neurosteroids were measured in dissected brain samples using LC-MS/MS. Behavioral assessment revealed that  $\beta$ -citronellol induced anxiety and memory impairment in zebrafish at all exposure concentration groups, and photosensitivity damage was observed at 1.0 mg/L exposure group. The result of neurosteroids, cortisol, which regulates stress and anxiety, increased in a concentration-dependent manner. Additionally, a significant decrease in progesterone and its metabolite allopregnanolone, was observed. Significant changes were also observed in neurochemicals related to the GABAergic system, dopaminergic system, and cholinergic system. Overall, in this study, we demonstrated that above a certain concentration of  $\beta$ -citronellol disrupts the balance between neurochemical systems and neurosteroids in zebrafish, leading to alterations in neurobehavior such as memory and anxiety. These results suggested that the high concentration of  $\beta$ -citronellol, which is mainly used in air freshener

products, must be well managed, and that an actual survey is needed to determine how much  $\beta$ -citronellol is contained in air freshener products.

#### **1.04.P-Tu012 Effects of Acute 6PPD-Quinone Exposure on Swimming Performance and Aerobic Metabolism in Juvenile Lake Trout (*Salvelinus namaycush*)**

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is a transformation product of the most widely used rubber tire antioxidant, 6PPD. Commonly found in road-way runoff, a variety of studies have reported this compound to cause acute lethality in several salmonid species at roughly  $\leq 1 \mu\text{g/L}$ , including lake trout (*Salvelinus namaycush*; 24 h  $\text{LC}_{50} = 0.51 \mu\text{g/L}$ ). However, there is currently a lack of research targeted at understanding sublethal toxicities of 6PPD-quinone exposure, particularly on swimming performance. Sensitive species show characteristic symptoms including gasping, spiraling, increased ventilation, loss of equilibrium and impaired swimming ability, suggesting a possible impact on cardiometabolic physiology. To evaluate potential effects of 6PPD-quinone on swimming performance and aerobic metabolism, juvenile lake trout were exposed to nominal aqueous concentrations of 0 or 0.5  $\mu\text{g/L}$  in a swim tunnel respirometer for 20 hours to assess temporal changes in standard metabolic rate (SMR) compared to unexposed controls. Following exposure, fish underwent a swim trial to determine critical swim speed ( $U_{\text{crit}}$ ), oxygen consumption ( $\text{MO}_2$ ), active metabolic rate (AMR), aerobic scope (AS) and energetic cost of transport (COT). After conducting the swim trial, fish were euthanized, and concentrations of muscle triglycerides and glycogen were determined. Preliminary data show that 6PPD-quinone exposure does not significantly impact oxygen consumption rates or energetic cost of transport during a critical swim test. In contrast, exposure appeared to cause a significant decrease in critical swim speed and active metabolic rate, providing evidence of altered metabolism. These results suggest that aqueous 6PPD-quinone exposure at environmentally relevant concentrations produces adverse effects that can diminish endurance and maximum swim speeds, which may affect survival of fish populations. This is the first study to analyze the toxicity of 6PPD-quinone on the swimming performance of fishes of commercial, cultural, and ecological importance and provides urgently needed information for environmental risk assessments of this emerging contaminant of concern.

#### **1.04.P-Tu013 Frontiers in Quantifying Wildlife Behavioural Responses to Chemical Pollution**

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Animal behaviour is remarkably sensitive to disruption by chemical pollution, with widespread implications for ecological and evolutionary processes in contaminated wildlife populations. However, conventional approaches applied to study the impacts of chemical pollutants on wildlife behaviour seldom address the complexity of natural environments in which contamination occurs. This presentation aims to guide the rapidly developing field of behavioural ecotoxicology towards increased environmental realism, ecological complexity, and mechanistic understanding. We identify research areas in ecology that to date have been largely overlooked within behavioural ecotoxicology but which promise to yield valuable insights, including within- and among-individual variation, social networks and collective behaviour, and multi-stressor interactions. Further, the review features methodological and technological innovations that enable the collection of data on pollutant-induced behavioural changes at an unprecedented resolution and scale in the laboratory and the field. In an era of rapid environmental change, there is an urgent need to advance our understanding of the real-world impacts of chemical pollution on wildlife behaviour. This review therefore provides a roadmap of the major outstanding questions in behavioural ecotoxicology and highlights the need for increased cross-talk with other disciplines in order to find the answers.

#### **1.04.P-Tu014 Evaluation of ecological toxicity for remediated soils contaminated with heavy metal using earthworm: Study on mechanism of toxicity manifestation**

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The firing range soil contaminated with heavy metals was remediated through a washing process. Heavy metal concentrations can be lowered below soil contamination standards, soil health deteriorated during the remediation process. Hence, there is a need for suitable techniques and methods to validate them for recovering the low-health remediated soil. The objective of this study was to explore techniques for recovering low-health contaminated soil and to verify the recovery levels using chemical and biological methods. The agents used in the recovery technology were vermicompost (biological agent), biochar (organic agent), and lime soil (inorganic agent). The soil mixed with the agents and remediated soil was aged while providing constant moisture for two weeks. The verification of soil health using earthworms utilized both OECD methods and the VISSET (Vibration Sensor for monitoring of Soil Ecological Toxicity) developed by the researchers. Earthworm survival rate, body weight change rate, and bioaccumulation concentration (BAF) methods were commonly applied, but earthworm behavior was additionally evaluated using the VISSET test method. The earthworm survival rate and weight variation in the remediated soil decreased significantly compared to the uncontaminated soil. This indicates a greater deterioration in soil health during the remediation process than during the contamination process. The results of earthworm activity also showed a significant increase in the remediated soil, indicating stress imposed on the earthworms. In soils treated with vermicompost and

vermicompost + lime soil, earthworm activity levels similar to uncontaminated soil, and it was confirmed that the level of chemical recovery was high. Conversely, in experiments utilizing biochar, lower chemical recovery levels were observed, and reduced earthworm activity was attributed to skin irritation. Since deterioration of soil health can occur not only during the contamination process but also during the remediation process, it is considered necessary to apply appropriate techniques to recovery the health of remediated soil for its reuse and recycling.

#### **1.04.P-Tu015 Toxicokinetic and behavioral effects of environmentally realistic microplastics**

*Andrew Barrick and Tham C. Hoang, Auburn University*

Plastics are ubiquitous substances with approximately 42,000 tons entering the environment annually. Upon entering the environment, plastics can be broken into micro(nano)plastics, particles with three dimensions <5mm, which increases their potential for bioaccumulation and trophic transfer. Plastics also contain hydrophobic contaminants. Micro(nano)plastics can potentially be novel vectors for biological uptake of these chemicals. Some plastics have neurotoxic additives such as phthalates, which can influence the behavior of exposed organisms. Alteration to behavior can influence predator-prey relationships and eventually cascade into ecological impacts. The relationship between plastics, neurotoxic additives, and environmental impact is poorly understood. Plastics are hydrophobic and are rarely neutrally buoyant, causing them to either float on the top of test chambers or sink to the bottom. This makes it difficult to establish dose-response profiles as the number of plastics introduced into a testing chamber is not equivalent to the number of plastics an organism will interact with. Therefore, it is necessary to understand the behavior surrounding consumption, excretion and retention of micro(nano)plastics to comprehend ecotoxicity. This presentation explores the toxicokinetics of micro(nano)plastic uptake and excretion, how it affects behavior, and how bioaccumulation relates to acute toxicity. Environmentally realistic micro(nano)plastics were prepared at <120µm by cyromilling commercially available plastic cups and forks. *Daphnia magna*, an aquatic crustacean, was exposed to plastic cup and fork particles following a dose-response format to determine acute toxicity and bioaccumulation. Both plastic fork and cup plastics were identified to be polyethylene and of similar sizes after cryogrinding, indicating that physical properties were comparable. Plastics were not acutely toxic towards *D. magna*. Marked differences in bioaccumulation and depuration between fork and cup particles were observed. Behavioral analysis demonstrated no change in localization of organisms within test chambers but demonstrated there may be slight alterations in how fast they moved. The study shows that minor changes in plastic composition, in particular color, can influence the uptake kinetics of micro(nano)plastics.

Keywords: behavioral effects, environmentally realistic plastics, *Daphnia magna*, dose-response relationships

#### **1.04.P-Tu016 Interactions between chronic pollution and behavioural variability within and among individuals of aquatic invertebrates.**

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Environmental pollution is often characterized by low-dose, chronic exposure regimes. Dose-response relationships of these types of exposure often fail to generate major effects on mean response, especially when utilizing conventional endpoints. We hypothesize that non-conventional endpoints like behaviour may capture more subtle effects of pollution and also, that variability in behaviour may be used to predict effects at population and community level. Variability may influence effects of pollution and vice versa, pollution may affect variability. These changes in variability may have profound effects on species interactions, and population dynamics, e.g. predator-prey interactions.

The aim of this project is to assess impact of low-dose chronic exposure of an antidepressant Fluoxetine and a metal Copper on behaviour and metabolism in populations of the freshwater isopod, *Asellus aquaticus*. First, we quantify within and among individual behaviour of wild populations of *A. aquaticus* and test the behavioural syndrome and pace-of-life hypothesis. Second, we test the effect of Fluoxetine and Copper on within and among variability of individual behaviour and metabolic rate. Third, we will test predictions generated on pollutant effects on the predator-prey dynamics in a mesocosm set-up with realized predation.

Here, we present results from the first step where behaviour and metabolic rate of wild-caught *A. aquaticus* was quantified without exposure to chemicals. We tested associations among behaviours i.e., behavioural syndrome, repeatability, and if behaviours were correlated with metabolic rate i.e. the pace-of-life hypothesis. We selected behaviours that strongly influence predator-prey interactions like activity, boldness/exploration and escaping behaviour. Activity and boldness/exploration showed a high repeatability and were positively correlated. *A. aquaticus* metabolism was a repeatable endpoint but was not correlated with any other endpoints measured.

This project is part of the CHRONIC project funded by EU under the Marie Skłodowska-Curie Actions, and the aim is to incorporate findings into Environmental Risk Assessments using low-dose chronic chemical exposure and non-conventional endpoints to identify sensitive ecosystems at risk.

#### **1.04.P-Tu017 Assessing the Behavioural Sensitivity of Gammaridae to Pesticides and Pharmaceuticals**

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Freshwater systems are continually exposed to various water streams, including municipal wastewater and agricultural runoff. Pollutants in the aquatic environments might change the behaviour of animals. Gammaridae are essential in marine and freshwater ecosystems as both decomposers, prey, and predators. Changes in their behaviour as a result of environmental pollution, may impact their ecological roles in the food chain.

In this study we aim to provide more insight in whether behavioural responses are potentially reliable forebodes for toxic effects in our aquatic environments. Therefore, we evaluate the sensitivity of swimming activity as an endpoint compared to mortality and immobility and compare how this sensitivity of swimming related endpoints differs between toxins with different modes of action.

In four laboratory experiments, we exposed the gammarid *Gammarus. pulex* to different compounds (two pesticides: imidacloprid (IMI) and chlorpyrifos (CPF), and two pharmaceuticals: carbamazepine (CBZ) and citalopram (CIT)) with different modes of action. After 48-h of exposure the dead and immobile organisms are counted, after which the swimming activity of all mobile organisms was individually assessed.

The immobility count was a more sensitive endpoint than mortality ( $EC_{50} < LC_{50}$ ) for IMI, CPF and CIT, while no mortality and immobility was observed in organisms exposed to CBZ concentrations up to 2000  $\mu\text{g/L}$ . Exposure to IMI, CIT or CBZ resulted in a decreased swimming speed and acceleration of *G. pulex* individuals, additionally, IMI exposure also resulted in a decreased thigmotaxis. No behavioural effects are observed after exposure to CPF, even though mortality and immobility occur at higher exposure concentrations. On the contrary, CBZ exposure that does not result in immobility or mortality, does influence the organisms swimming behaviour.

The four compounds all have a different ratio between traditional endpoints (mortality and immobility) and swimming behaviour endpoints. The sensitivity of swimming activity and its relation to mortality and immobility differs much between the four tested pollutants. The behavioural endpoints are therefore not a reliable forebode of lethal effects, but these differences in sensitivity can give more insight in the mode of action of these pollutants in *G. pulex*.

#### **1.04.P-Tu018 Review: Opportunities and Limitations of Invertebrate Behaviour as Relevant Endpoint in Ecotoxicological Testing**

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Invertebrates are important model organisms in ecotoxicological testing as they occupy key positions in food webs of terrestrial and aquatic ecosystems. Next to the classical endpoints of OECD testing guidelines, environmental relevant chemicals can affect invertebrate behaviour. Behavioural alterations may lead to effects on population level traits, e.g. a reduction in foraging, reproduction, migration or predator avoidance. Therefore, the resulting impacts may affect populations and ecosystems in general function.

Examples for terrestrial invertebrates used as ecotoxicological model organisms are *Apis mellifera*, *Folsomia candida* and *Eisenia andrei* as well as *Daphnia magna* and *Lymnaea stagnalis* as aquatic model organisms. Invertebrates include a wide range of species of terrestrial and aquatic organisms. Much data is available on the ecology of most invertebrates, but often, little is known of the effects of chemicals (pesticides, pharmaceuticals, ...) on the behaviour of invertebrates. The OECD guidelines for the testing of chemicals (Section 2: Effects on Biotic Systems) mostly address lethality/immobility, size, hatching, emergence or reproduction and do not consider relevant behavioural endpoints. Behavioural endpoints, whose affection might lead to population-relevant impacts, are phototaxis, locomotion, rheotaxis, activity time and predator avoidance behaviour, among others.

As mentioned above, alterations in behaviour can occur for many parameters and have effects on diverse population-relevant traits. If the vertical migration of daphnids was affected due to chemical exposure, they would not be able to respond to predator presence and be an easy prey. If the swarming behaviour of *Chironomus riparius* was time-shifted by behavioural alterations from chemical exposure, the mating might occur for fewer pairs, leading to a shrinking population. If the defensive behaviour of *Lymnaea stagnalis* was affected, they might not or slower withdraw into their shell and therefore have a raised risk of predation.

The common ecotoxicological model organisms and also non-standard invertebrates shall be examined with regard to their ecology and their suitability for behavioral observations. Literature was reviewed and evaluated based on the suitability, opportunities and limitations for behavioural endpoints in different invertebrates. Existing guidelines are discussed with regard to the possibilities of integrating behavioral endpoints in a regulatory environment.

#### **1.04.P-Tu019 Chronic exposure of environmentally relevant concentrations of the SSRI sertraline impact feeding and behaviour in the freshwater worm Tubifex tubifex**

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Pharmaceuticals, like antidepressants, have been identified as potential threats to aquatic ecosystems due to their widespread and increasing use, and incomplete removal during wastewater treatment. This study focuses on the potential behavioural effects of the SSRI sertraline, on aquatic invertebrates. These effects, observed at environmentally relevant concentrations, encompass physiological and behavioural endpoints, highlighting the need for non-conventional measures that align with the intended mode of action. Antidepressants, designed to modulate neural and behavioural processes, may induce subtle changes that conventional endpoints might overlook. Incorporating behavioural bioassays, assessing indicators like movement patterns and feeding habits, offers a more comprehensive understanding of the ecological impacts of pharmaceuticals. This inclusion is crucial for identifying and quantifying sublethal effects, safeguarding ecosystem health.

Addressing the limited information on sertraline's impact on behavioural change in sediment-based ecosystems, our study evaluated the chronic toxicity of sertraline on *T. tubifex*. Assessing feeding rate, oxygen consumption, and behavioural changes, we found that sertraline concentrations correlated with reduced feeding rates, increased locomotion, and delayed light avoidance in *T. tubifex*. This research enhances our understanding of sertraline's ecotoxicity in sediment environments, emphasizing the importance of considering behavioural endpoints in environmental risk assessments.

#### **1.04.P-Tu020 Effects Of Copper And Cadmium, Isolated And In Mixture, On The Behavior Of The Copepod *Eurytemora Affinis***

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Metals are ubiquitous in the environment and anthropogenic and natural activities can increase their amounts. Most likely they end up in the aquatic systems, where they can interact among themselves and with other contaminants (e.g., microplastics, bisphenols, pharmaceuticals) resulting in complex mixtures that can affect biota. Copper (Cu) is an essential metal for organisms, while cadmium (Cd) is considered a non-essential metal, which contribute to the challenge of studies dealing with Cu-Cd mixtures. Copepods are an important link between primary producers and higher trophic levels, being sensitive organisms in ecotoxicology studies. *Eurytemora affinis* (Copepoda, Calanoida), which is very representative from estuaries was collected in the Seine estuary (Tancarville, Normandie, France), and were exposed to Cu (20 µg L<sup>-1</sup>), Cd (30 µg L<sup>-1</sup>) and their mixtures (CuCd) during 24 h, in addition to control. Velocity of the organisms was evaluated using DanioVision/Ethovision. After 6 min of acclimation in dark conditions in the chamber, 48 organisms per condition were assessed during one cycle of 2 min dark followed by one cycle of 2 min light. Data were evaluated using LMEM (Linear Mixed Effect Model), GLMM (General Linear Mixed Model) and GAMLSS (Generalized Additive Model for Location, Scale and Shape) models in time bins of 10 sec and 2 min. The best adjustment for our data was obtained using the GAMLSS model with ZAGA (zero adjusted gamma) distribution and the time bin of 10 sec. In the time bin of 10 sec, the results indicate a higher activity of the organisms according to the time, with a great inter-individual variance, independent of the treatment. The organisms were more active in light, with a slightly higher velocity in Cu and CuCd treatments, but without significant differences compared to control. For the time bin of 2 min, the organisms were more active in light and with higher velocity in Cu and CuCd treatments. These data indicate how sensitive it is to deal with behavioral analysis since the variance obtained with the organisms can be significant and the use of different time bins can result in different outputs. In addition, we recommend the use of different statistical models to evaluate the responses and to obtain a better data adjustment.

#### **1.04.P-Tu021 Drugs in a Noisy World: A Dangerous Combination**

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The close proximity of freshwater habitats to urban centres poses direct threats to fish through discharge of chemicals, pharmaceuticals and other wastes. While the effects of a small number of high profile pharmaceuticals are well documented, aquatic habitats can be contaminated with dozens of different chemicals. Simultaneously, human activities generate noise which can penetrate freshwater ecosystems and disturb natural behaviors. While there has been a gradual increase in awareness of the harmful effects of noise on wildlife almost no study has investigated the interactions of sound and chemicals on fish behavior. In this study we exposed zebrafish to either the antibiotic amoxicillin at either 0.2 or 20 µg/L or the anti-inflammatory diclofenac at 0.004 or 4 µg/L, or a combination of both, for 28 days. The behavior of the fish was then measured using a series of standardized behavioural and shoaling tests in the presence and absence of an anthropogenic noise (motor boat). Prior exposure to both drugs affected the fish's response to noise, including time to emerge in a boldness test, time to reach food and habitat use. Importantly, for future research we noted the order of noise exposure in each test was important. In some cases we observed different outcomes dependent on whether the fish were exposed to control conditions or noise in the first test. Increasingly fish live in a multi-stressor world that includes both chemicals and physical stressors. As the effects of human activities penetrate even the deepest oceans we need a better understanding of the harmful effects of noise

#### **1.04.P-Tu022 Heat Stress Makes Antibiotics More Dangerous to Zebrafish**

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The widespread use of antibiotics for humans and livestock has led to widespread contamination of surface waters. Beta-

lactam (amoxicillin) is one of most frequently used antibiotics. After administration more than 80% is eliminated from the body, ultimately ending up in the environment with low biodegradability and high toxicity.

Due to climate change aquatic species are increasingly facing temperature challenges. Temperature is critical for maintaining organism homeostasis including metabolic rate and body function. Thermally stressed fish use heat shock proteins (HSP) as chaperones to mitigate the influence of heat stress through binding to proteins. Increased expression of HSPs is an indicator of temperature stress. Several studies found that HSP70 and HSP90 genes are key biomarkers of thermal stress.

When we combine stressors of different natures simple rules of additive and synergistic effects may not apply. In the present study we measured the effects of environmentally realistic concentrations of amoxicillin at two different temperatures on zebrafish. We analysed the effects of these stressors on fish shoaling behavior and the fish's response to a predator alarm cue. We also measured levels of heat shock proteins and the composition of the gut microbiome. We hypothesize that: 1) The impact of amoxicillin will be greater at high temperature, 2) Both amoxicillin and high temperature will alter the composition of the gut microbiome, and 3) Both temperature and amoxicillin will inhibit zebrafishes response to predation cues

The results show a strong effect of heat stress the gut microbiome ( $p \leq 0.0001$ ), with no effect of treatment ( $p = 0.495$ ) or sex (0.862). All the three exposure factors: amoxicillin, conspecific alarm and heat affected shoaling behavior. In nature fish must respond and adapt to multiple natural and anthropogenic stressors. We found that temperature had a strong effect on the gut microbiome but there was surprising little effect of amoxicillin. On the other hand both temperature and amoxicillin affected fish behavior, as well as the way that the fish responded to an alarm chemical. We live in a complex world. Ecotoxicological studies should reflect this complexity in their design.

#### **1.04.P-Tu023 Neurotoxicity Assessment in Adult Danio rerio using a Battery of Behavioral Tests in a Single Tank**

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The presence of neuropathological effects proved to be, for many years, the main endpoint for assessing the neurotoxicity of a chemical substance. However, in the last 50 years, the effects of chemicals on the behavior of model species have been actively investigated. Progressively, behavioral endpoints were incorporated into neurotoxicological screening protocols, and these functional outcomes are now routinely used to identify and determine the potential neurotoxicity of chemicals. Behavioral assays in adult zebrafish provide a standardized and reliable means to study a wide range of behaviors, including anxiety, social interaction, learning, memory, and addiction. Behavioral assays in adult zebrafish typically involve placing the fish in an experimental arena and recording and analyzing their behavior using video tracking software. Fish can be exposed to various stimuli, and their behavior can be quantified using a variety of metrics. The novel tank test is one of the most accepted and widely used tests to study anxiety-like behavior in fish. The shoaling and social preference tests are useful in studying the social behavior of zebrafish. This assay is particularly interesting since the behavior of the entire shoal is studied. These assays have proven to be highly reproducible and sensitive to pharmacological and genetic manipulations, making them valuable tools for studying the neural circuits and molecular mechanisms underlying behavior. Additionally, these assays can be used in drug screening to identify compounds that may be potential modulators of behavior. We will show in this work how to apply behavioral tools in fish neurotoxicology, analyzing the effect of methamphetamine, a recreational drug, and glyphosate, an environmental pollutant. The results demonstrate the significant contribution of behavioral assays in adult zebrafish to the understanding of the neurotoxicological effects of environmental pollutants and drugs, in addition to providing insights into the molecular mechanisms that may alter neuronal function.

#### **1.04.P-Tu024 Analysis of sleep/wake cycles in zebrafish larvae**

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Zebrafish larvae are a model organism increasingly used in the study of the effect of neuroactive chemicals on vertebrate sleep/wake cycles. Sleep disturbances have a negative impact on mood, cognition and overall health. Here we present a protocol to assess over 24 h sleep/wake cycles in zebrafish larvae subjected to 12 hr light/dark periods in 48-well plates, using video-tracking technologies. The protocol can be used to determine if the exposure to environmental pollutants or drugs can lead to sleep disturbances. The results on the effect of the tire rubber-derived 6PPD-quinone on zebrafish sleep/wake cycles presented here demonstrate the suitability of using this protocol in fish neurotoxicity studies. This protocol provides a new relevant tool to be used in the pharmacology and toxicology fields.

Simultaneous determination of sleep/wake cycles in 48 zebrafish larvae

The determination of sleep/wake state is based on mobility and immobility bouts

#### **1.04.P-Tu025 Chronic Exposure of Bis(2-ethylhexyl) Phthalate Accelerates the Development of Depression Caused by Chronic Stress**

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Depression is a psychiatric disorder that leads to suicide at a high rate, and a positive correlation between depression and

exposure to environmental pollutants is suspected. In previous studies, we reported that long-term exposure of bis(2-ethylhexyl) phthalate (DEHP) causes depression in a mouse model. DEHP-induced depression occurs by inhibiting glutamatergic neurotransmission through disruption of glutamate (Glu)-glutamine (Gln) homeostasis in the medial prefrontal cortex (mPFC), which is like to the mechanism of chronic stress-induced depression. To investigate the correlation between DEHP exposure and chronic stress in the development of depression, we exposed mice to low levels of DEHP, subjected them to a level of stress that did not cause depression, and observed changes in depressive behaviors and biomarkers of depression. As a result, changes in depressive behaviors and biomarkers were observed in DEHP-exposed and stressed mice, but these changes were not shown in mice DEHP-exposed or stressed mice alone. In DEHP-exposed and stressed mice, the behaviors indicating anxiety, despair and anhedonia were observed, and levels of corticosterone and ROS/RNS were increased. In addition, the disruption of Glu-Gln cycle such as suppressed glutamine synthetase activity and decreased Glu and Gln levels was revealed in mPFC. These results prove that the development of depression can be accelerated by the synergy of exposure to environmental pollutants and chronic stress and notice the need for a new approach in studying the human toxicities of environmental pollutants in the future.

#### **1.04.P-Tu026 Studying behavior and physiological stress in fish: a case study on the effect of artificial light at night on cortisol excretion and accumulation of thinlip mullet (*Chelon ramada*)**

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Urban ecosystems are submitted to multiple anthropogenic stresses, which impact aquatic communities. Among those urban stressors, artificial light at night (ALAN) affects the aquatic biota. The wavelengths penetrating below the surface and the residual light intensity after diffraction and reflection on it vary according to the physical and biological characteristics of the water (salinity, density, suspended matter, including biological particles, e.g., plankton). All the characteristics of light play a role in the biology and physiology of all the species studied, including fish. The scientific literature highlights species-specific differences in the effects of ALAN on fishes. Furthermore, while the literature on the impact of nighttime illumination on urban biodiversity is growing, studies on fish behavior remain rare. In this work, we used thinlip mullet (*Chelon ramada*, Mugilidae), a common species widely distributed in European urbanized aquatic environments, as a biological model. Thinlip mullet is a rare representative of the herbivore/detritivore trophic guild in the ichthyological fauna, and as such, plays an essential role as a vector of energy between the benthic and pelagic compartments of aquatic food webs. An experiment was set up to understand the effects of ALAN on the behavior and physiological stress of thinlip mullet under controlled conditions. A total of 15 fish were exposed to different light modalities to mimic natural or altered photoperiods in an urban context during four successive trials of 72 hours each. The "control" aquaria received daytime light exposure, followed by no exposure during the night hours, and with gradual day/night transitions to mimic a "natural" photoperiod. The "exposed" aquaria received a continuous light during both day and night and with either direct or indirect modalities, to mimic an "altered" urban photoperiod. Fish behavior was analyzed with video imaging (Ethovision; variables including distance between subjects, movement velocity, distance traveled). In parallel, physiological impacts were assessed by measuring cortisol concentrations in the water and in fish blood at successive time steps. Results are currently being analyzed, but preliminary findings suggest significant differences in swimming and schooling behaviors of fish between the control and exposed conditions, while cortisol response (in the water and in fish blood) appears similar in all conditions.

#### **1.04.P-Tu027 Larval White Sturgeon Behavior Affected by Copper**

**Holly J Puglis, Robin Calfee and Edward E Little, U.S. Geological Survey (USGS)**

Early life stage white sturgeon are sensitive to copper with adverse behavioral responses observed during previous studies. The objectives of the present study were to quantify the effects of copper exposure on white sturgeon swimming behaviors and determine their time to response. Larval sturgeon (1 day post hatch) were exposed to copper (0.5-8 µg/L) for 14 days. Abnormal behavioral changes were observed within the first few days of exposure including loss of equilibrium and immobilization. Digital video tracking software revealed decreased swimming activity with increasing copper concentration. Significant changes in behavior and mortality occurred at concentrations of copper between 1-8 µg/L. Effect concentrations were calculated for all responses on Day 14. The 20% lethal concentration (LC20) was 4.36 µg/L Cu and the 20% effect concentration (EC20) for loss of equilibrium/immobility (LOE/IM) was 2.13 µg/L Cu. Swimming activity EC20s ranged from 1.30–1.50 µg/L Cu. Water quality criteria normalized for the test water chemistry using the biotic ligand model was less than the LC20 but greater than the EC20 for LOE/IM and swimming activity endpoints. Our results indicate that behavioral endpoints were more sensitive than some standard toxicity test endpoints and can be effectively expand the sensitivity of standard toxicity tests for white sturgeon. Swimming behavior was impaired to the extent that survival in the field would likely be jeopardized. Such data would provide managers a useful metric for characterizing the risks of copper contamination to white sturgeon.

#### **1.04.P-Tu028 Low Doses of Antidepressants Impair the Visual Motor Response Behavior of Zebrafish (*Danio rerio*) Embryos – a Red Flag for Developmental Neurotoxicity**

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Venlafaxine, paroxetine, and sertraline, widely used and increasingly prescribed antidepressants, are gaining increasing attention as pollutants in the aquatic environment. Their presence in water bodies is due to excretion by humans and incomplete removal during wastewater treatment. These pharmaceuticals, which have been developed to interfere with

neurotransmitter homeostasis in humans at low doses, have the potential to affect aquatic organisms such as fish, as the nervous system of vertebrates is fundamentally similar.

When characterizing the acute effects of these compounds on the developing zebrafish embryo using the Fish Embryo Acute Toxicity (FET; OECD TG 236) test, pericardial edemata, jaw deformation, fin seam degradation and balance disturbances during swimming were observed. At EC<sub>10</sub> concentrations, behavior was examined in more detail using the visual motor response (VMR) assay, which allows a direct investigation of swimming behavior and the reaction to light-dark transition stimuli. The data showed reduced activity in both, swimming behavior and response to the stimulus.

In summary, the results of the experiments indicate a disruption of key behavior patterns in early life-stages, which in turn may be an indicator of developmental neurotoxicity. Considering the environmental concentrations already present in European waters and the increasing trends in annual prescription rates, these pollutants can pose a realistic threat to the aquatic environment.

#### **1.04.P-Tu029 Testing if and how municipal wastewater effluent affects fish habitat choice in the wild**

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Wastewater treatment plants (WWTPs) are one of the largest point-sources of nutrient and chemical pollution to freshwater habitats around the world. Mounting evidence shows that WWTP effluents can have detrimental effects on aquatic organisms. And yet, research has shown that fish are still abundant near WWTPs in the wild. This raises an important question: are WWTPs an ecological trap for aquatic wildlife? Ecological traps attract animals, but expose them to sub-optimal conditions facilitating local population declines. To begin to test the hypothesis that WWTPs act as ecological traps, we evaluated whether fish are behaviourally attracted to wastewater effluents in the wild. We measured fish habitat choice and residence time at a WWTP outfall site in Sweden using acoustic telemetry. We deployed an array of acoustic receivers spanning 2 km up- and downstream from the wastewater outfall to track fish movement and habitat choice. We collected and tagged 80 fish of 4 different species representing two prey species (Bream, Rudd) and two predator species (Zander, Northern pike; we tagged 20 fish of each of the four species). We measured their movements after release over 6 months, from May – Oct 2023. We predicted that if fish can detect and are attracted to the effluent (indicating that WWTPs may be an ecological trap), then they will spend longer near the outfall, than in the up- or downstream habitats. Contrarily, if fish are unable to detect the effluent, then we expect fish to distribute themselves along the acoustic receiver gradient with no detectable attraction to the outfall site. We will relate our findings to water quality data and contaminant loadings measured along the river. Although there is mounting concern about the effects of WWTPs on aquatic animals, we still lack data on how long animals reside in effluent impacted habitats. This study will help us understand the extent to which fish are exposed to effluents and the contaminants within them.

#### **1.04.P-Tu030 Zebrafish embryos as model for behavioral toxicology assessment of pharmaceuticals**

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The use of pharmaceuticals has increased in recent years. Since wastewater treatment plants are not efficient in removing these compounds, a fraction are released into aquatic ecosystems, posing concern for both environmental and human health concern. Indeed, the 4th WatchList (Under the Water framework directive) identified some pharmaceuticals like cytostatic drugs as substances of concern.

In Our study, we used zebrafish embryos to evaluate the acute effect of 8 pharmaceuticals, 5 cytostatics (5-fluorouracil, cisplatin, cyclophosphamide, imatinib and dasatinib) and 3 anticonvulsants (topiramate, valproic acid and carbamazepine) on larvae behavior. The use of zebrafish embryos in toxicology represents an alternative strategy aligned with the 3Rs principles, seeking to reduce the impact of experimental procedures on animal welfare. Furthermore, given the substantial costs and prolonged assessments associated with developmental toxicology, zebrafish has emerged as a preferred model organism for conducting high-throughput developmental toxicity studies. Zebrafish embryos were exposed from 6 hpf to 120 hpf and at the end of the exposure, mortalities and effect on behavior were assessed. Behavior was evaluated by using the Light/Dark Transitional test with an acclimatization period followed by a range of light and dark intervals. Substances such as topiramate and 5-fluorouracil did not show mortality or effect on behavior. On the other hand, changes in behavior were observed in other substances such as valproic acid and cyclophosphamide, in the case of valproic acid (LC<sub>50</sub> 18.40 μM) showed hypoactivity at 27 μM.

The outcome of our study provides valuable information on the effect of pharmaceuticals in aquatic organisms. Moreover, our results support the use of zebrafish embryos as an alternative to the animal models for the screening and assessing toxicity of candidate compounds for regulatory acceptance. It also lays the foundations for a faster and reliable environmental and human risk assessment based on New approach methodologies (NAMs).



#### **1.04.P-Tu031 The antidepressant paroxetine reduces intraspecific behavioural variance in zebrafish**

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The presence of distinct personality traits (e.g., bold and shy) among individuals and within a population is a vital component of a survival strategy to optimise an adaptive response to environmental stressors including drug pollution. Paroxetine (PAR) is a selective serotonin reuptake inhibitor (SSRI) antidepressant widely detected in surface waters (up to 270 ng/L) and in fish tissues. Several studies demonstrated the ability of SSRIs to interfere with fish behaviour however, effects on personality/coping styles, which is strictly associated with behaviour, remain largely unknown.

In this study, zebrafish embryos (3hpf) were submitted to acute and chronic exposures to PAR to assess potential effects on personality-associated behavioural responses. In the acute exposure, organisms were exposed up to 48 hpf to 0 µg/L, 0.4 µg/L and 40 µg/L and allowed to recover in clean media up to 45 dpf, whereas, in the chronic exposure, embryos were exposed up to 45 dpf to the same concentrations of PAR (0 µg/L, 0.4 µg/L and 40 µg/L). At 45dpf fish from all experimental groups were submitted to group and individual-based behavioural tests to separate and rank fish according to their personality pattern (bold and shy). The basal swimming activity of bold and shy fish under light and dark conditions was analysed using the ZebraBox automated tracking system (Viewpoint, Lyon, France), considering the total swimming distance (TD, mm) and the total time of inactivity (%).

The obtained results revealed that under control conditions, bold fish are more active than shy fish (higher swimming distance and lower inactivity time) in dark and light conditions. However, exposure to PAR disrupted this personality-associated behavioural pattern, with bold fish adopting a similar behaviour to shy individuals (decreased swimming distance and increased time of inactivity in comparison with controls) in both light conditions (light and dark). The acute exposure in a key susceptibility window (from 3hpf to 48hpf) to a low concentration of PAR (0.4 µg/L) induced similar effects to those elicited by chronic exposure to a higher concentration (40 µg/L). These results, particularly those observed for the acute exposure in the first 48hpf, require further studies to explore the underlying mechanisms and the potential intergenerational and transgenerational implications of PAR on behavioural phenotypic variation in fish.

#### **1.04.P-Tu032 Understanding Anxiety Behavior in Developing Zebrafish (*Danio rerio*) Exposed to Environmental Toxicants**

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The environment is facing an ever-growing input of substances that interact with the nervous system. Neurotoxicity has been found to be the most prevalent toxic mode of action in European surface waters and it is estimated that up to 30% of commercially used substances may act neuroactive. To assess the impact of these chemicals to non-target ecological receptors of concern (i.e. fish), a fundamental understanding of the mechanistic processes leading to altered behavioral phenotypes is required. One neurotoxic outcome that has received limited consideration to date is anxiety, despite its obvious impacts on the fitness of affected organisms and populations, such as feeding, mating and predator avoidance.

The AnxioTox project aims to address this knowledge gap by studying anxiety in fish, improving our understanding of the specific mechanisms leading to anxiety-like behavior and establishing a robust line of evidence across different levels of biological organization. Using a set of environmentally relevant substances known to modulate anxiety-like behavior in fish through different neurotoxic modes of action, we will characterize anxiety-like behavioral alterations of zebrafish as a representative model species across different developmental stages up to 8 days post fertilization (dpf). Preliminary experiments investigating the effects of the anxiolytic buspirone and the anxiogenic caffeine on zebrafish locomotion have identified the light/dark transition test, in combination with other endpoints including thigmotaxis, as a promising tool for discriminating anxiety-like behavior in early developmental stages (5 dpf). We will repeat these experiments with other anxiety modulators and verify the method with suspect substances not previously associated with anxiety. Optimized data analysis and quantification will facilitate detailed description of movement patterns, creation of holistic locomotor profiles and thus enable sensitive detection of alterations. Furthermore, we will assess social avoidance behavior by evaluating the response of an exposed organism to conspecifics.

By studying the anxiety-like behavior in zebrafish, this project will contribute to a better understanding of how exposure to chemicals of environmental concern affects normal behavioral patterns. This is essential to efficiently design experimental setups, to increase confidence in such experiments, and to promote future standardization of behavioral assays with zebrafish larvae.

#### **1.04.P-Tu033 Short-Term Exposure to Median Avoidance Concentration of Chlorpyrifos Induces Behavioral and Physiological Consequences in *Poecilia gillii* fish**

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Chemical contamination has the potential to influence the habitat selection of organisms. Chemicals can either repel, attract, or confuse an organism, thereby influencing their decision to remain in or flee from a particular habitat. In scenarios where animals are exposed to discharges of contaminants and must decide whether to tolerate or avoid them, can they experience physiological consequences during this habitat selection process? To explore this, we assessed the avoidance response of the tropical fish, *Poecilia gillii*, to the organophosphate insecticide chlorpyrifos (CPF) using a non-forced open gradient exposure system. Once the AC50 (median avoidance concentration) was calculated and, then, fish were forcedly exposed to that concentration (AC50) for a duration of 3 hours, matching the avoidance test period. We then assessed signs of physiological and behavioral effects, including changes in brain and muscle cholinesterase activity (ChE), respiratory frequency, and swimming patterns. During the avoidance test, the fish exhibited a random distribution throughout the open gradient system during the initial period of the test, displaying a clear avoidance response only after 3 hours of exposure, with an AC50 of 18.6 (14.8 – 25.6) µg/L. The forced 3-hour exposure of *P. gillii* to their CPF AC50 resulted in significant ChE inhibition in their brains and alterations in their swimming patterns. The exposure eliminated their side preferences in the aquarium and increased their presence in the upper part of the water column. Our findings contribute to the understanding of the immediate effects that fish may experience during the rapid process of recognizing and avoiding of neurotoxic contaminant in the event of a discharge of contamination. These physiological and behavioral effects can potentially impact the fitness and stability of the population.

#### **1.04.P-Tu034 Development of a High Throughput Thigmotaxis Assay to evaluate Neurotoxicity in Zebrafish Embryos**

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Neurotoxicity (NT) of commercialized chemical substances is mainly studied directly following guidelines 424 and 426 of the OECD that include behavioral endpoints in rodents such as activity, learning, and memory but do not include endpoints related to anxiety. Moreover, rodent studies are time-consuming, costly, and present ethical issues regarding animal welfare. There is therefore a need for the development of new approach methodologies (NAMs) to evaluate the potential neurotoxicity of thousands of chemicals on the market in a high throughput human relevant manner. The present work will be done within the European Partnership for the Assessment of Risks from Chemicals (PARC), subtask 5.2.1e, that has the objective of developing and evaluating NAMs to address NT. In this context the zebrafish embryo (ZFE) up to 120 hours post fertilization (hpf) is an *in vivo* model with high human homology but not included in animal welfare regulations and considered a NAM. Our objective is to develop a high throughput thigmotaxis assay to evaluate possible effects on anxiety-like behavior of neurotoxic chemicals in 120 hpf ZFE. The assay will include anxiety-like responses to visual and acoustic stimuli. Results of assay development, including responses to known anxiogenic, anxiolytic, and putative negative substances, and comparisons between 24 round and 96 square well plate formats will be presented. When completed and evaluated for performance with a suite of neuroactive chemicals, this assay could be included in a second-generation developmental NT and a first-generation adult NT alternative test batteries.

#### **1.04.PC Behavioural Toxicology: Methodologies and Research Needs**

##### **1.05 Better Alignment of New Approach Methodologies and Adverse Outcome Pathways to Support Next Generation Risk Assessment**

##### **1.05.T-01 The Cross-Species Applicability of the Thyroid Hormone System Disruption (THSD) AOP Network and its Utilisation in Cross-species Extrapolations**

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Thyroid hormone system disruption (THSD) is considered a human and environmental health issue. Consequently, multiple efforts are underway to develop fast and reliable test methods to detect compounds causing THSD. These efforts aim to reduce rodent-based testing strategies by introducing new approach methodologies (NAMs) like zebrafish embryos. Under the current EU legislation, zebrafish embryos are not protected up to five days post fertilization. Further, the hypothalamic-pituitary-thyroid axis of vertebrates is highly conserved and thus zebrafish embryos could be employed as *in vivo* NAMs to support the extrapolation of THSD effects to mammals. These extrapolations can be supported by the adverse outcome pathway (AOP) framework as it provides information about the linkages between a molecular initiating event (MIE) and an adverse outcome (AO) via causally linked key events (KEs).

By combining single THSD AOPs, developed in different species, an AOP network is formed for which we previously assessed the cross-species applicability. This cross-species AOP network is the basis of our case study in which we explore the use of zebrafish embryo test as NAMs for extrapolations of THSD-effects to mammals. First, the response of the (zebra)fish and mammalian thyroid hormone (TH) system to THSD model compounds (methimazole, 6-propyl-2-thiouracil, perchlorate, iopanoic acid) was investigated qualitatively and focusing on MIEs, KEs and AOs. Comparing these results to the outcome of

the tDOA evaluation supports the relevance of KEs and AOs linked e.g., to impaired neural development or eye development for cross-species extrapolations. Secondly, we compare TH levels of exposed/unexposed mammals and (zebra)fish to determine whether TH level alterations in both taxa are comparable in direction and magnitude. Baseline TH levels were established and the impact of THSD model compounds is currently evaluated. Based on preliminary results it appears that e.g., methimazole-induced decreases of T4 levels are similar in rats and fish.

Our work highlights the potential of the THSD AOP network to support the extrapolation of THSD effects across vertebrate taxa, thereby promoting the use of alternative test methods. Specifically, our ongoing comparison of TH level alterations will facilitate this effort and help bridging THSD effects between mammals and (zebra)fish.

#### **1.05.T-02 Bridging the gap between human toxicology and ecotoxicology via the development of Cross Species Adverse Outcome Pathway: a case study on silver nanoparticles**

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Historically, toxicological and ecotoxicological risk assessment of stressors were conducted separately. But recent efforts have been made in both disciplines to reduce animal testing and develop predictive approaches instead, e.g., via conserved molecular markers, and in vitro and in silico approaches. Among them, Adverse Outcome Pathways (AOPs) have been largely adopted to facilitate the prediction of molecular toxic effects to larger biological scales. Thus, more and more, toxicological data are used to inform on ecotoxicological risks and vice versa. An AOP has been previously developed that summarized all the available data on *Caenorhabditis elegans* (AOP 207) and predicted reproductive toxicity of silver nanoparticles (AgNPs) via oxidative stress. In the current study, we aim to extend the taxonomic domain of applicability (tDOA) of this AOP. Various types of data including in vitro human cells, in vivo, molecular to individual, etc. from our previous studies have been collected and structured into a new cross species AOP network that can be used for both human toxicology and ecotoxicology risk assessment. The first step of our approach was the assessment of endpoints from selected studies to fit the AOP criteria. For each study, the endpoints were listed and translated into AOP wiki Key Events (KE) terms. The second step aimed to extend tDOA using the SeqAPASS web-based tool on each KE and for the overall AOP. Cross species extrapolation enabled to predict the reproductive toxicity of AgNPs for over 1000 species. This approach shows that various types of data can be integrated into an AOP framework and thus facilitates access to knowledge and prediction of toxic mechanisms without the need for further animal testing. Next steps will focus on expanding this approach to literature data, for example by combining it with systematic review approaches, and the extrapolation of tDOA for KE with no specific target receptor, using other approaches than SeqAPASS, e.g., phylogenetic approaches. To go further, quantitative data can also be assessed to build a quantitative AOP.

#### **1.05.T-03 Explainable Artificial Intelligence Models for Developmental and Reproductive Toxicity Prediction using ToxCast Data in Adverse Outcome Pathway Framework**

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Artificial intelligence (AI) models offer a new opportunity to assess the potential toxicity of a vast amount of environmental chemicals. However, many toxicity prediction models are inherently 'black boxes', making interpretation challenging for toxicologists and hindering the regulatory acceptance of these models. Inherently, the mechanisms leading to the onset of apical toxicity are complex, and in the absence of process evidence, it is difficult to trust the results, which may be, in the worst case, a mere coincidence. The adverse outcome pathway (AOP) framework holds promise in addressing this issue. AOPs can link molecular toxicity endpoint to human and ecotoxicity endpoint. In response, this study aims to develop explainable artificial intelligence models for predicting developmental and reproductive toxicity using ToxCast data within an AOP framework. Initially, in vitro bioactivity data from ToxCast and in vivo toxicity data from eChemPortalDB and ECOTOX DB were gathered. ToxCast database offer extensive and publicly accessible molecular toxicity information for thousands of chemicals. These data were then integrated into AOPs associated with developmental and reproductive toxicity, and each assay-AOP pair underwent further assessment to determine relevance to taxa within ecosystems. Finally, machine learning models were developed to predict each mechanistic and regulatory endpoint-based toxicity. As a result, models for each key event and apical endpoint achieved high performance, with AUC-ROC values ranging from 0.56 to 0.88. To broaden the applicability of models to both human and ecotoxicology contexts, further analysis of the taxonomical domain of applicability (tDOA) of AOP-based prediction models is underway. This study can serve as a guide for developing explainable machine learning models based on AOPs, addressing the current primary bottleneck in using AI models for chemical risk assessment.

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#### **1.05.T-04 A quantitative adverse outcome pathway (qAOP) linking mitochondrial uncoupling to growth inhibition in *Lemna minor***

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Uncouplers are a group of chemicals that can cause uncoupling of oxidative phosphorylation (OXPHOS) in mitochondria,

leading to mitochondrial dysfunction and various adverse effects in organisms. The study aims to construct a quantitative adverse outcome pathway (qAOP) based on the OECD-endorsed AOP (AOPwiki# 263) for ecological risk assessment. Using the OECD TG 221 protocol (Lemna sp. Growth Inhibition Test), tailored laboratory studies were conducted to generate a matrix of data capturing temporal and concentration-dependent changes of four key events (KE1446, KE1771, KE1821, and KE1521) in *Lemna minor* exposed to the model uncoupler CCCP (carbonyl cyanide m-chlorophenyl hydrazone). The KEs were quantified at 48h, 96h, and 7d using the TMRM assay, ATP luminescent assay, EdU assay, and length/size measurement, respectively. Multiomics analysis (transcriptomics + metabolomics) provided mechanistic insights into possible feedback/feedforward loops influencing the quantitative understanding. The results indicated that after 7-day exposure, CCCP induced concentration-dependent reductions in OXPHOS, ATP, cell proliferation, and growth in *L. minor*, with increasing EC<sub>50</sub> values of 0.49, 1.98, 2.18, and 2.75 μM, respectively. Such increased EC<sub>50</sub> values not only demonstrated sensitivity of these endpoints to CCCP, but also supported dose-response concordance and possible causal relationships between these KEs. Analysis of results from 48h and 96h is ongoing. Subsequently, the qAOP models were built using two approaches, piecewise structural equation modeling (PSEM) and Bayesian network (BN). Besides quantification of response-response relationships, both approaches were capable of identifying the most influencing KE(s) in the pathway and evaluating the predictive ability of the qAOP. Results demonstrated the initial qAOP model presented good predictability of the AO from MIE. The approaches used herein can be easily adopted by other studies for de novo development of qAOP models.

#### **1.05.T-05 Navigating Complex Biological Systems with PBPK, Text Mining and AI: in silico NAMs for the Development of Reliable and Robust Quantitative Adverse Outcome Pathways**

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In the context of this study, a computational methodology is presented. It starts with environmental exposure and concludes with the a disease by constructing qAOPs. Numerous tools and methodologies are developed encompassing NLP, AI, exposure, PBTK, and systems biology models. Commencing with exposure scenarios, the exposure is quantified across the three routes. Pharmacokinetic models are employed to convert external exposure into internal exposure. To delve into the molecular level, in vitro experiments to generate untargeted metabolomics and transcriptomics data were conducted. The data were integrated to generate multi-omics, aiding in the identification of metabolic pathways perturbed following internal exposure. This data was input into MINER, a text mining toolbox developed to convert representations of KEGG pathways into systems biology models. The incorporation of as many biological pathways as possible resulted in the construction of a big systems biology model, consisting of 1348 differential equations. The kinetics of the model were parameterized with data originating from the BRENDA and SABIORK databases as well as with the use of Deep Learning models to estimate the enzyme properties of interest. Data derived from the HMDB database were utilized to initialize the model. In addition, a methodology was developed using ML and GANs to initialize the model. An ML model was generated for each endogenous metabolite, irrespective of whether its concentration was known. This was conducted for validation purposes. Subsequently, the model was executed twice. The second run also incorporated the fold change results obtained from the omics. The simulation output unveiled metabolites whose concentrations changed by an order of magnitude. Owing to this circumstance, publicly available were gathered to scrutinize the variations in concentrations when an individual is impacted by a disease. The mathematical equation describing the endogenous metabolite provides a comprehensive network of interactions involving metabolites, genes, and proteins. For the AOP development, a bottom-up approach is employed, leveraging our knowledge of the AO and the available information about the MIE. By utilizing transcriptomics, conducting network analysis, and performing a literature review, the precise MIE was determined. Utilizing the developed NLP tools, the AOP was established. Additionally, experimental studies were identified for the quantitative description of KERs.

#### **1.05.P Better Alignment of New Approach Methodologies and Adverse Outcome Pathways to Support Next Generation Risk Assessment**

##### **1.05.P-Th001 AOP-Network: Investigation of a Crosstalk Between Retinoic Acid Signaling and Thyroid Hormone Signaling During Zebrafish Early Development.**

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As realistic scenarios involve exposure to environmental mixtures of chemicals, characterizing regulatory mechanisms during embryonic development is crucial for a more realistic hazard assessment. This study contributes to a novel Retinoic Acid signaling (RA) AOP network (AOPN) and to a better mechanistic understanding of existing Thyroid Hormone (TH) signaling AOPN. It investigates the crosstalk between RA and TH signaling during zebrafish early development.

Both RA and TH signaling pathways play a major role in various early developmental processes. RA, known as a morphogen, influences anteroposterior patterning, organogenesis, craniofacial, spine, and tail development. And TH signalling plays a significant role in the development of structures such as the anterior and posterior swim bladder, and eye development (AOPs #155-159 and #363 respectively). Our data indicate that RA signaling disruption may also affect posterior swim bladder inflation and eye development in zebrafish embryos, suggesting a potential crosstalk between RA and TH signaling. The potential crosstalk could be either direct, meaning that RA will affect TH signaling, or indirect meaning that both signaling pathways regulate similar developmental processes and need to be balanced to lead to normal development.

To explore this hypothesis, zebrafish embryos were exposed and co-exposed to All-Trans Retinoic Acid (ATRA) and TH Receptor antagonist (Diclazuril) during critical developmental windows (4-48hpf, 4-72hpf, and 4-120hpf). An assessment of malformations associated with each signaling pathway was performed. Craniofacial and tail malformation at 120hpf were indicative of RA signaling disruption, and eye malformation (changes in retinal pigment epithelium morphology) at 120hpf and posterior swim bladder inflation at 170 hpf were associated with TH signaling disruption. To further investigate TH disruption, we used a zebrafish transgenic line (thyroglobulin – mCherry) expressing a fluorescent signal in thyroid follicles. Finally, gene expression analysis at 15hpf focusing on key genes related to TH and RA signaling disruption, and the wnt and Sonic Hedgehog (shh) pathways, was performed. Both wnt and shh pathways are known for their role in cell migration during development and were identified as key players in developmental processes leading to observed malformations.

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### **1.05.P-Th002 Quantitative Systems Modeling of Adverse Outcome Pathways as Translational Tools in Human Health Risk Assessment**

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Adverse outcome pathway (AOP) is a mechanistic representation of a causal chain of critical toxicological effects at diverse biological organizations ranging from the initial (chemical) stressor-target interaction to an apical or population adverse outcome. Along with developing different AOPs for predicting human health risk, *in vitro* effect assays can provide mechanistic evidence about novel key events (KE) and possibly fill data gaps. In practice, individual *in vitro* effect assays often use human cell lines to provide information about subsets of molecular or cellular KEs. To enhance biological coverage, we propose cross-systems modeling to integrate *in vitro* and *in vivo* data to offer mechanism-based toxicity prediction.

First, we develop quantitative systems modelling to advance quantitative understanding of key event relationship (KER) as outlined by OECD guidelines about AOPs, namely about response-response relationship, time scale, modulating factor, as well as feedback loops. We demonstrate detailed systems modelling of chemical-induced activation of cellular stress pathways using public data. Second, we apply systems modelling on apical hepatotoxicity endpoint by integrating *in vivo* biomarkers. For both cases, we also exemplify an integration of systems modelling of AOP with biokinetics, which inform chemical fate and distribution *in vitro* or *in vivo* setups. Besides, we create platforms to interactively visualize model predictions for the two examples, which aims to facilitate the modelling update for potential users without modelling experience.

To conclude, systems modelling of AOPs can integrate various types of data to increase coverage of biological mechanisms to better assess chemical-induced human health risk. A broader perspective of systems modelling of AOP for non-chemical stressors will also be discussed.

### **1.05.P-Th003 Unveiling an Adverse Outcome Pathway for *Caridina africana* Following Exposure to Nanodiamonds.**

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Nanomaterials, characterized by having at least one dimension within the range of 1 nm to 100 nm, hold promise as emerging technologies for various applications. Nanodiamonds (NDs) have unique properties that make them appealing for use in the medical field. However, once introduced into aquatic environments, nanomaterials can have adverse effects on the resident organisms. To elucidate the underlying mechanism, these effects can be unravelled through the adverse outcome pathways (AOPs) construct. Adverse outcome pathways outline the sequence of events by which contaminants affect organisms, from molecular-level changes, progressing to physiological, individual, and population-level consequences. While AOPs have been established for many toxicants, they are relatively scarce for nanomaterials. This study aimed to fill this gap by constructing an AOP on the effects of NDs on the freshwater shrimp, *Caridina africana*. *Caridina africana* was exposed to NDs at sublethal concentrations, and the metabolomic, respiration rate, heart rate as well as behavioural responses were determined. Exposure of *C. africana* to NDs resulted in an increased level of Proline metabolites, an elevated oxygen consumption rate and reduced behavioural activity. These responses of *C. africana* was a result of increased physiological stress, which in turn led to an increased mortality rate. Although NDs are considered inert, its toxicity is attributed to its particle size, agglomeration potential, and the adsorption to the shrimp's external surface. These toxicity attributions generated reactive oxygen species which affected the shrimp's physiological responses. These hierarchical biological responses were successfully integrated into the AOP construct, which provides valuable insight into the mechanism by which nanomaterials can impact aquatic organisms.

### **1.05.P-Th004 Development of Quantitative Adverse Outcome Pathway for Respiratory Diseases Induced by CMIT/MIT using Transcriptomic Profiling**

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Respiratory diseases linked to humidifier disinfectant (HD) exposure, particularly involving the biocide mixture 5-chloro-2-methyl-4-isothiazoline-3-one and 2-methyl-4-isothiazolin-3-one (CMIT/MIT), have raised significant health concerns in Korea. Despite established epidemiological connections between HD exposure and respiratory diseases, the precise toxicity mechanisms remain elusive. This study aimed to construct a quantitative adverse outcome pathway (AOP) for CMIT/MIT-induced respiratory diseases, employing transcriptomic profiling. First, differentially expressed genes (DEGs) analysis

revealed a dose-response relationship for the three time points (4, 24, 72h) using BMDEExpress2 ( $|\text{Log}_2\text{FC}| > 0.6$ ,  $P\text{-value} < 0.05$ ). Subsequently, toxicity pathways were identified using Ingenuity Pathway Analysis (IPA) ( $P\text{-value} < 0.05$ ) and the Benchmark Dose (BMD) medium value were calculated for each pathway. We selected toxicity pathways with a time- and dose-response relationship and linked them to four respiratory diseases based on epidemiological findings. This study supported the early toxicity pathways by analyzing transcriptome data from established AOP based on epidemiological studies conducted at CMIT/MIT. Also, our study proposes a quantitative AOP for CMIT/MIT through the time- and dose-response relationship of transcriptome data, highlighting the understanding of toxicity mechanisms by quantifying early key events in respiratory diseases following CMIT/MIT exposure.

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Keywords: Biocides, Adverse Outcome Pathway, Transcriptome, Benchmark Dose, Respiratory Diseases

### **1.05.P-Th005 Development of Quantitative Adverse Outcome Pathway Leading to Hepatocellular Carcinoma using Transcriptomics Data with Benchmark Dose Analysis**

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Hepatocellular carcinoma (HCC) is the fourth most common cancer globally and is the second leading cause of cancer death in East Asia. Allyl alcohol is an allylic alcohol used as a precursor in the manufacture of plasticisers, pharmaceuticals, resins, acrolein, and herbicides. Allyl alcohol undergoes alcohol dehydrogenase oxidation to form acrolein leading to hepatotoxicity and eventually HCC. An adverse outcome pathway (AOP) is a framework that integrates existing knowledge to describe the effect of a substance from its initial interaction with a biological system (molecular initiating event) cascading through the different levels of cellular, linked together by key event relationships required to produce a toxic effect (adverse outcome). In this study, we present an AOP for HCC using transcriptomic data and benchmark dose (BMD) analysis to identify the pivotal point at which key events in the AOP occur. We extracted human gene expression data of allyl alcohol exposure from Open TG GATEs database, and calculated the BMD and mapped the values onto genes and biological processes to identify the important processes initiated at these values. Disease and biological process data were collected from the Comparative Toxicogenomic Database to build the AOP. We found that the pathways leading to HCC were time and dose sensitive. We suggest an AOP leading to HCC via “upregulation of FGFR3”, “upregulation of IGF1R”, “activation of P13K cascade”, upregulation of mitotic G2-G2/M phases”, and “cell proliferation and survival”. This study provides a framework to suggest the adverse outcomes of environmental toxins through integrating publicly available multi-omics data. Furthermore, benchmark dose analysis can be used to predict the pivotal point at which key events in the AOP occur.

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Keywords: transcriptomics, quantitative adverse outcome pathway, benchmark dose, allyl alcohol, hepatocellular carcinoma

### **1.05.P-Th006 Development of adverse outcome pathways and testing strategies for endocrine disrupting chemicals and mixtures promoting metabolic dysfunction-associated steatotic liver disease**

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common liver disorder found in a quarter of the world's population. Growing evidence suggests that exposure to endocrine disrupting chemicals (EDCs) can promote the initiation and/or progression of MASLD, either directly or by exacerbating the effects of a high-fat diet, genetics and/or lifestyle factors. The newly launched **Horizon Europe project EDC-MASLD (2024-2029)** aims to elucidate the impact of EDCs on the initiation and progression of MASLD, utilizing cutting-edge systems biological/toxicological approaches. One of the missions of EDC-MASLD is to develop adverse outcome pathways (AOP) and integrated testing strategies for rapid identification and assessment of EDCs promoting MASLD. New AOPs will be assembled based on a collection of existing knowledge through systematic review, relevant key events and relationships in the AOPWiki, in-house data, and systems biological understanding (e.g., genome-scale metabolic model) of the progression of MASLD. The strength of evidence support for the AOPs will be assessed based on the Evolved Bradford Hill weight of evidence considerations, according to OECD's guidance document on AOP development. The chemical applicability domain will be defined by identifying EDCs affecting the same MIE or KEs in the MASLD AOPs, based on existing information from screening programs such as the Tox21. Bioinformatics approaches will be used to identify similarities of major genes and proteins across biological systems and define the biological applicability of the AOPs. In addition, quantitative AOPs will be constructed for selected chemicals. The AOPs will suggest critical knowledge needs, relevant chemical domain, test doses/concentrations, key events for

measurement and exposure duration for the EDC tests with different experimental models. As an alternative to the mammalian models, zebrafish (*in vitro* hepatic 3D spheroids and *in vivo* transgenic embryos) will be used as a pre-screening tool to select candidate EDCs and mixtures (as suggested by human cohort studies) for in-depth assessment. Combined effect modeling will be performed with the screening data to identify possible interactive effects of EDC mixtures on the progression of MASLD. The project will generate comprehensive knowledge on the chemical-mediated progression of MASLD and supply useful tools for future regulation of EDCs.

#### **1.05.P-Th007 Alterations of ubiquitin-dependent catabolic pathway and growth in the brackish water clam *Corbicula japonica* caused by environmental stress in estuaries revealed by transcriptome analysis**

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Estuaries are dynamic ecosystems that experience environmental impacts, such as saltwater intrusion and the influx of anthropogenic and natural resources, including heavy metal pollution and hazardous substances. The brackish water clam (*Corbicula japonica*) is constantly exposed to stressful salinity gradients and high levels of heavy metals in the freshwater-saltwater interface of estuary environments. To identify the key molecular pathways involved in the response to salinity changes and heavy metal bioaccumulation, we obtained the transcriptomes of *C. japonica* inhabiting different salinities and heavy metal distributions in Gwangyang Bay (Korea) using RNA sequencing. Among a total of 404,486 assembled unigenes, 5534 differentially expressed genes were identified in *C. japonica* inhabiting different conditions, 1549 of which were significantly upregulated and 1355 were significantly downregulated. Correlation analyses revealed distinct gene expression patterns between the low and high conditions of salinity and heavy metal bioaccumulation. Functional annotation revealed significant downregulation of genes involved in “ubiquitin-dependent protein catabolic process,” “tricarboxylic acid cycle,” and “intracellular protein transport” in *C. japonica* from the high condition compared to the low condition. Transcription and translation pathways were significantly enriched in the high condition. Additionally, upon comparison of the low and high conditions by qRT-PCR and proteasome enzyme activity analyses, our findings demonstrated that environmental stress could suppress the ubiquitin-proteasome complex (UPC). Additionally, transcriptomic changes under high salinity stress conditions may be related to an increase in cellular protection by defense enzymes, which leads to more energy being required and a disruption of energy homeostasis. Ultimately, this could cause growth retardation in the clam *C. japonica*. In summary, this study provides the first evidence of UPC suppression induced by a combination of high salinity and heavy metal bioaccumulation stress in *C. japonica*, which could compromise the survival and growth of estuarine bivalves.

#### **1.05.P-Th008 Ecotoxicogenomic Hazard Assessment of Artificial Sweeteners in Aquatic Model Organisms**

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Since their approval as food additives, artificial sweeteners are commonly used in food, beverages as well as in personal care products all around the world. The widespread and intensive consumption of artificial sweeteners in combination with their high stability and water solubility has led to their release into the aquatic environment, where they prove to be persistent. Given that no detailed environmental risk assessment was carried out as part of the food additive approval process, it is still unclear whether and to what extent ecotoxic effects are to be expected. Although this has already been pointed out in literature multiple times, studies regarding the ecotoxicity of artificial sweeteners are still lacking. The few studies that have been carried out, could already reveal some ecotoxic effects, such as neurotoxicity in zebrafish embryos caused by Acesulfame K or increased immobility of daphnids caused by Sucralose.

With this in mind, the aim of this project is to enable an assessment of the ecotoxic potential of artificial sweeteners, using OMIC-methods. To fill the existing data gaps, investigations regarding their effects on ecotoxicologically relevant model organisms from different eukaryotic kingdoms will be performed. More precisely, the aquatic plant *Lemna minor*, the Crustacean *Daphnia magna* and the teleost fish *Danio rerio* (embryo) will be examined. In addition to the performance of the corresponding guideline of the Organisation for Economic Cooperation and Development (OECD), effects will be recorded at the gene expression level using RNA sequencing and thus enable insights into the modes of action that correspond to the hazardous effects. This promising combination of methods will be performed on artificial sweeteners for the first time, which finally may show that the assessment of ecotoxicity should no longer be neglected in the approval procedures for food additives.

#### **1.05.P-Th009 Omics-based fingerprinting of neurotoxic and microtubule-disrupting pesticides in zebrafish embryos**

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Plant protection products play a pivotal role in agriculture, yet their impact on non-target organisms and the environment raises growing concerns due to some of their non-selective harmful effects and bioaccumulation potential. Conventional environmental risk assessments of pesticides primarily focus on lethal concentrations, neglecting the intricate influence of sublethal effects and exposure timing during critical developmental phases, potentially leading to developmental abnormalities. This study employs transcriptomic analysis to uncover the molecular fingerprints of two pesticides – the neurotoxic insecticide

endosulfan and the microtubule assembly inhibitor herbicide benfluralin - in zebrafish embryos. In the present study, zebrafish embryos were exposed to different sublethal concentrations of endosulfan and benfluralin in a modified 96 hours zebrafish embryo toxicity test (zFET). Notably, no significant effects on survival or hatching rates were observed compared to the control group. However, transcriptomic analysis revealed distinct molecular responses in embryos exposed to each pesticide, delineating their specific impacts on biological processes. Benfluralin exposure led to differential expression of genes associated with muscle cell differentiation, heart contraction, and skeletal muscle development. Conversely, endosulfan exposure targeted genes crucial for hormone response, neuron apoptotic processes, and neuron death, pivotal for nervous system development. These findings highlight the intricacy of sublethal effects induced by these compounds on non-target organisms, emphasizing the need for a more comprehensive assessment beyond survival-focused evaluations. Integrating these molecular insights into risk assessment frameworks is imperative, especially in establishing transcriptomic points of departure (tPODs) protective against chronic effects. This integration recognises complexities beyond traditional endpoint measurements and provides additional weight-of-evidence for a more holistic assessment of pesticide toxicity.

#### **1.05.P-Th010 In vitro assessment of the synergistic potentials of the ingredients in surface applied preservatives for dermal cytotoxicity and skin sensitization**

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Wood preservatives are biocidal products used to protect wood used in building materials, furniture or consumer products. Consumers may be exposed to low concentrations of biocidal components on the skin through direct daily use of the products. Taking into account the dermal route of exposure, we attempted to determine the cytotoxicity and skin sensitization of single components and their mixtures contained in commercial wood preservatives. Three preservatives were selected because their ingredient information was available in a previously established biocidal product database in South Korea. As a first step, nine compounds were tested for their cytotoxicity using the HaCaT, skin keratinocytes. The compounds with EC<sub>50</sub> values less than 1,000  $\mu$ M were included in binary or tertiary equitoxic mixtures, depending on whether these compounds were simultaneously present in the same preservative product. The EC<sub>x</sub> value derived from the mixture toxicity test was compared with the EC<sub>x</sub> value predicted by the concentration addition (CA) model to calculate the model deviation ratio (MDR) to determine the interaction between mixture components. We found that certain combinations showed synergism in the cytotoxicity assay, so as a further step we tried to confirm the mode of action and synergistic effects by the ARE-Nrf2 assay using KeratinoSens<sup>TM</sup> cell line based on OECD TG 442D. In the cytotoxicity test, five compounds showed EC<sub>50</sub> values of less than 1,000  $\mu$ M, and a synergistic effect was observed with an MDR of 7.28 to 25.03 in a mixture containing a carbamate family compound. In the KeratinoSens assay, a synergistic effect was also observed in a carbamate compound containing mixtures. We confirmed that the synergistic interaction of a carbamate group biocidal compound was mediated by the second key event (KE) of the AOP (Adverse Outcome Pathway) for skin sensitization. Given the relatively high synergistic effect, further research and consideration of the hazards and interactions of the mixtures in commercial products is needed for the safety management of wood preservatives and related biocidal products.

#### **1.05.P-Th011 In vitro Toxicity screening of real-life mixture using HepG2 cells**

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To develop the risk prediction technology for mixture toxicity, a reliable and extensive dataset of experimental results is required. In everyday life, people are exposed to complex mixtures comprising more than ten compounds unintentionally, yet most published literature has only data in combinations with two or three substances. Therefore, there is a very limited dataset for predicting the toxicity of complex mixtures. On the other hand, complex mixtures may have different mode of actions (MoA) due to their variety of composition, posing difficulty in the prediction using conventional toxicity prediction models, i.e., concentration addition (CA) and independent action (IA) models. In this study, we aimed to generate an experimental dataset comprising complex mixtures. These mixtures were representative of our real-life exposure to be served as reliable dataset for developing the prediction model for mixtures with different MoA. To identify the target complex mixtures, we referred the findings of the HBM4EU project. We identified three groups of 7~10 substances that were commonly detected together in human bodies, namely environmental phenols, perfluorinated compounds, and heavy metal compounds, assuming them to have different MoA. Additionally, we added a separate mixture consisting of 9 organophosphate flame retardants (OPFRs) which may have a similar MoA. All target substances were tested for cytotoxicity using HepG2 cell lines and the EC<sub>10</sub> values were derived after 48-hrs exposure. Then, 50 different complex mixtures were generated with equitoxic mixtures of EC<sub>10</sub> levels, randomly selected. To determine the interaction effect, the model deviation ratio (MDR) was calculated by comparing the observed EC<sub>10</sub> with the predicted EC<sub>10</sub> from the CA model. These values were then categorized to the three types of interactions. Dose-response curves and EC<sub>50</sub> values were calculated for all complex mixtures. Out of 50 mixtures, none demonstrated synergism, while 6 mixtures exhibited antagonistic effect (MDRs 0.31~0.48). The remaining mixtures showed additivity (MDRs 0.50~1.34). Our experimental data has been formatted and constructed to the database. It will be used to conduct further research that aims to develop a novel two-stage mixture prediction model for application in the mixture risk assessment.

#### **1.05.P-Th012 Apical and molecular endpoints in zebrafish embryos exposed to cadmium**

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The EPIBOOST project aims at validating the epigenetic modifications caused by chemicals as biomarkers of relevance which can support more accurate environmental risk assessment. For this, a multiparametric approach is pursued by looking at effects of chemicals from the individual and biochemical levels to the transcriptomic and epigenetic levels, with the aim of establishing a link between phenotypic and molecular endpoints. In this work, taking cadmium (Cd) as case study, 96 hours zebrafish larvae were exposed to sublethal concentrations of Cd for 24 hours. Apical endpoints analysed included mortality, development, and swimming behaviour (swimming distance and path angles). At the same time, we analysed biochemical endpoints including biotransformation enzymes (glutathione-s-transferase (GST)), antioxidant enzymes (catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR)), oxidative damage markers (lipid peroxidation (LPO)), and neurotoxicity markers (acetylcholinesterase (AChE)). At concentrations tested no effects on survival or development (delays or anomalies were observed) and the antioxidant system did not seem to have been activated (no changes in GST, CAT, GPx or GR activities were detected). AChE was however inhibited suggesting neurotoxicity elicited by the tested Cd concentrations. This was further supported by changes in behavioural parameters at the last concentration tested namely changes in the proportion of rapid/slow movements and changes in the proportions of zigzag movements/straight movements. At present, we are performing transcriptomic and DNA methylation analysis to evaluate the effects of Cd exposure at the genomic level. In the near future we will integrate molecular and apical endpoints to identify biomarkers of exposure to cadmium. This data will contribute to understand the Cd adverse outcome pathway and validate epigenomic effects as a valuable tool to understand effects of contaminants at larger ecological scales.

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Keywords: *Danio rerio*, embryo development, locomotor behaviour, biomarkers, transcriptomics, DNA methylation

#### **1.05.P-Th013 Molting inhibition in non-model species *Calanus finmarchicus* exposed to aquatic veterinary agents**

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The Norwegian aquaculture industry is one of the most important industries in coastal areas and farmed Atlantic salmon (*Salmo salar*) represents the second largest export in Norway. Control of threats to this economically important industry is important at both local and national levels. One of the largest threats to this industry is parasite infections of salmon from salmon louse (*Lepeophtheirus salmonis*). Chemotherapeutics and other strategies have been used to protect farmed fish in open-net pens from this parasite, one of them being the use of chitin synthesis inhibitors (CSI) such as teflubenzuron (TEF) and emamectin benzoate (EMB). TEF and EMB are among the most used feed-administered CSI. Although the adverse endpoints are similar, the compounds have different molecular modes of action. TEF inhibits the expression of the enzyme chitin synthase causing reduced chitin production and disrupted molting in immature stages of salmon louse. EMB on the other hand, has a neural inhibitory effect on the regulation of ecdysis causing the same endpoint as TEF. Thus, both these CSI have adverse effects on the molting process in salmon louse and are especially effective against the more molting frequent larval and pre-adult life stages. As chitin synthesis is an arthropod-specific process, CSI has low toxicity to vertebrates and algae. However, they are likely to have adverse effects on nontarget arthropod species such as the copepod *Calanus finmarchicus* a key species in the Northern Atlantic ecosystem. The aim of this project is to assess the sensitivity of *C. finmarchicus* to CSI exposure and uncover the causal relationships between CSI exposure and adverse effects. The objectives are to study CSI-mediated changes in chitin content, expression of genes related to molting (ecdysis), and imaging of structural deterioration of the copepod cuticle. Based on this information, a conceptual overview linking events together in relationships in an adverse outcome pathway (AOP) framework will be outlined. Preliminary results indicate that nauplii of *C. finmarchicus* display increased mortality at concentrations >200 times lower for teflubenzuron than emamectin benzoate, thus *C. finmarchicus* are much more sensitive to the teflubenzuron.

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#### **1.05.P-Th014 A Gamechanger In The Mutagenicity Characterization of Azo Dyes**

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Azo dyes, characterized by the functional group (-N=N-), are the most widely used dye class in several applications such as textiles, cosmetics, pharmaceuticals and food. That is why studies on their mutagenicity have been carried out over the years. However, the uncertainty about their purities, solubility issues, and lack of molecular confirmation may have compromised

some of the published results. Well-chemically characterized samples are mandatory in the development of high quality *in silico* prediction models. The Salmonella/microsome assay is the most common and easy way to evaluate chemicals mutagenicity, using different genetically modified strains of *Salmonella* bacteria to detect gene mutations. In the literature, the use of TA98 and TA100 strains has been considered enough to detect the mutagenicity of the majority of chemicals. The strain YG1041 has shown higher sensitivity to azo dyes. In a recent publication, from six azo dyes evaluated, two would not be considered mutagenic if not tested with this strain. But YG1041 has not been included in any regulatory testing battery so far. This study aimed to verify the effect of the inclusion of YG1041 in the mutagenicity evaluation of five highly purified azo dyes (purity > 97%) selected from the North Carolina State University's Maax Weaver Library (MWDL). Chemical structures and purity were confirmed using a High-performance liquid chromatography quadrupole-time-of-flight mass spectrometry (HPLC-Q-TOF MS) equipped with an electrospray ionization (ESI). Dyes were tested with YG1041, TA98 and TA100 with and without metabolic activation (rat liver S9 5%, Aroclor induced) in a miniaturized version of the Salmonella/microsome assay, the microplate agar (MPA). Dyes were dissolved in dimethyl sulfoxide at their solubility limit and tested in concentration-response experiments. The five dyes showed mutagenicity to YG1041 with S9, but only one was also mutagenic with TA98. None was mutagenic to TA100. The inclusion of YG1041 was crucial to detect the mutagenicity of 4 of the five dyes tested. Along with the literature data, and the results obtained in this work we believe that the YG1041 should be considered by regulatory agencies for azo dyes mutagenicity evaluation.

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### **1.05.P-Th015 Insights into the Microbial Degradation Potential of Hydrocarbon Contaminants across the Baltic Sea Environment**

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The Baltic Sea is loaded with petroleum-based substances, e.g., hydrocarbons and plastic waste, from various sources. Microbial communities drive organic matter degradation, and are naturally capable of degrading environmental pollutants. The information on the degradation potential of contaminant-exposed microbial communities can be used to estimate persistence or to predict the fate or accumulation of petroleum-based pollutants in the environment. This would be useful in managing and mitigating the crisis in crude oil and plastic pollution in the Baltic region. However, there is still limited information on the degradation potential of microbial communities in the Baltic Sea, alongside the lack of environmental monitoring information on these contaminants. This prompts the need for a comprehensive profile of the microbial degradation capacity of the region. In this study, we utilize metagenomics and profile the microbial degradation potential of the Baltic Sea, specifically focusing on hydrocarbon aerobic degradation in its benthic ecosystem. We report the genes and enzymes involved in alkane, alkene, aromatic, and plastic aerobic degradation, and biosurfactant production across Baltic Sea regions, and identify key environmental factors influencing gene diversity. Our report aims to deepen our understanding of microbial degradation of contaminants that would be useful for improving mitigation strategies for crude oil spills and plastic pollution in the Baltic Sea while addressing critical environmental concerns.

### **1.05.P-Th016 Differential expression of different clusters of non-coding RNAs (ncRNAs) and altered gene regulation in response to exposure of ten environmental stressors to EndoC-BH1 pancreatic b - cells**

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Non-coding RNAs (ncRNAs) play a crucial role in epigenetic alterations after exposure to environmental chemical factors. ncRNAs include the microRNAs (miRNAs), the long non-coding RNAs (lncRNAs), small nucleolar RNAs (snoRNAs), and among others include also the pseudogene transcripts which are RNA molecules that have been transcribed from a DNA segment that resembles a protein-coding gene but, this DNA is never expressed as a protein. In the context of the OBERON Horizon2020 European project ten different environmental stressors including BPA, BPF, BPS, BP, DBP, CdCl<sub>2</sub>, PFOS, PFOA, DEHP, and DDE in various concentrations ranging from 0.1 to 100 nM, and to timepoints ranging from 24 to 72 hours. Afterwards, cells were subjected to RNA extraction and whole genome microarray analysis was performed using the Agilent SureScan platform. Bioinformatic analysis was conducted applying two different methods, the Agilent GeneSpring software and the limma R package. Subsequent analysis was focused to identified differentially expressed genes (DEGs) that encode for lncRNAs and pseudogenes that have been found to all treated concentrations for each different chemical. Microarray probes are updated and originate from the eArray database and include identified or predicted lncRNAs, snoRNAs and pseudogenes. Results have shown that in all the treated cells with environmental stressors differential expressed lncRNAs regulate genes associated to DNA repair, modulation of chromatin function, interference with signalling pathways, and nucleotide excision repair. In addition, snoRNAs which affect cell and tissue pathophysiology were found to be significantly altered in all concentrations in DDE, DEHP, DBP and BPA treated cells. All the aforementioned ncRNAs ultimately affect gene expression in diverse biological and physiopathological contexts. Thus, since EndoC-βH1 cell line is a model for human beta cells, the next step is on the hand to validate the differentially expressed ncRNAs as novel biomarkers of exposure linked to insulin secretion, proliferation, apoptosis, and endoplasmic reticulum (ER) stress, and on the other hand to develop Adverse Outcome Pathways

(AOPs) with the final endpoint being different conditions of the metabolic syndrome, such as insulin resistance, type 2 diabetes, obesity and non-alcoholic fatty liver disease (NAFLD).

#### **1.05.P-Th017 An overview of the effects of Polycyclic Aromatic Hydrocarbons (PAHs) mixtures in both in vivo and in vitro models.**

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Polycyclic aromatic hydrocarbons (PAHs) are complex carbon and hydrogen compounds formed by the fusion of aromatic rings and are commonly found in the environment. Due to their low water solubility, PAHs persist, bioaccumulate, and induce toxic effects (oxidative stress, inflammation, and cell death). Some PAHs, pose challenges as they exist in complex mixtures in the environment. The main objective of this work is to understand the mode of action (MoA) of environmentally relevant PAH mixtures using several biological models, like fish primary hepatocytes, zebrafish immortalized hepatocytes (ZFL cell line), immortalized human hepatocytes (HepG2 cell line) and adult fish.

In the *in vitro* experiments cells were exposed to three PAHs (Benzo[a]pyrene - B[a]P, Phenanthrene - Phe and Benzo[b]fluoranthene - B[b]F) individually and in mixtures (1:1, 1:2, 2:1). Toxicological responses, including viability, CYP1A1 expression and activity, and DNA damage, were assessed after 24h and 48h in a concentration range from 5 to 100 µM. For the *in vivo* bioassay, *Sparus aurata* specimens were exposed for 42 days to Phe and B[a]P individually and in mixtures (1:2, 2:1). Biochemical analyses related to oxidative stress and PAH detoxification pathways were conducted.

Results from the *in vitro* assays showed that B[a]P upregulated CYP1A1, enhancing activity and inducing genotoxic DNA damage. Mixtures of Phe and B[a]P exhibited heightened CYP1A1 mRNA levels and DNA damage. In the *in vivo* bioassay, was found that in adult *S. aurata*, PAH mixtures showed different effects on antioxidant enzymes and CYP1A1 expression compared to individual compounds. However, no significant increase in DNA damage or lipid peroxidation was observed.

Overall, this study demonstrates the unpredictable behavior of mixtures. These findings highlight the underestimated risk posed by PAH mixtures. Therefore, efforts should be made to improve environmental guidelines, as the possible synergistic or antagonistic effects of mixtures of different types of PAH may be underestimated.

#### **1.05.P-Th018 Deriving Predicted No-effect Concentration of Tributyltin for Marine Environment Using Taxon-specific Adverse Outcome Pathways**

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Tributyltin (TBT), an acknowledged endocrine disrupting chemical (EDC), can trigger endocrine disruption in marine organisms, resulting in adverse outcomes of growth and reproductive effects. The marine ecological risks posed by TBT compounds remain a concern following the global ban. However, conventional procedures for the derivation of predicted no-effect concentration (PNEC) typically fail to account for non-apical endpoints for endocrine disrupting effects and diverse taxonomic groups, which may provide insufficient protection for marine species. In this study, a method for PNEC derivation of EDCs was constructed applying taxon-specific adverse outcome pathways (AOPs) and species sensitivity distribution. Chronic toxicity dataset for indigenous marine species containing apical endpoints as well as non-apical endpoints associated with taxon-specific AOPs were employed to derive TBT's PNEC for Chinese marine environment. Sensitive non-apical endpoints included craniofacial malformations (fish; growth), morphology alteration of appendages (crustaceans; growth), ecdysteroid levels (crustaceans; growth), vas deferens sequence index (mollusca; reproduction), levels of sex hormones (fish; reproduction), and developmental stage of testicular cells (fish; reproduction). According to the sensitivity discrepancy analyses, the sensitivity of fish, crustaceans, mollusca, and microalgae to TBT decreased sequentially. The reproduction had a lower hazardous concentration for 5% of species (0.693 ng/L) in comparison to the growth effect (1.53 ng/L). The PNEC based on combined apical and non-apical endpoints was 1.3 ng/L (95% confidence intervals: 0.23~7.1 ng/L), which was 2.4 times lower than that estimated on the basis of apical endpoints. Imposed data from field studies on gastropods highlighted the significance for supplementation of non-apical endpoints to improve the conservativeness of PNEC. The current results will provide a valuable reference for deriving PNEC of EDCs with ecological relevance and low uncertainty, protecting marine organisms from endocrine disruption.

#### **1.05.P-Th019 Modes of Action of Thyroid Disruption: Insights from Zebrafish Transcriptomics**

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Chemicals released into the environment pose a significant threat to wildlife species. Traditional approval processes, especially for detecting endocrine-disrupting compounds, involve costly and ethically challenging animal experiments. This study explores an alternative approach, utilizing transcriptome analysis in zebrafish embryos to identify gene biomarkers for thyroid-related modes of action (MoA) with the aim to potentially serve as screening tool for compound prioritization. Four reference

substances, iopanic acid (IOP), methimazole (MMI), propylthiouracil (6-PTU), and triiodothyronine (T3), all targeting thyroid pathways, were examined. Zebrafish embryos were exposed to different concentrations of all test substances following OECD guideline 236. Physiological endpoints and low sub-lethal concentrations for the main study were determined. The main test involved RNA sequencing and differential gene expression analysis at 96 hours post fertilization. Survival and swim bladder length reduction were observed in embryos exposed to IOP and MMI, with delayed hatching observed only for MMI. Transcriptome analysis identified 973 differentially expressed genes (DEGs) for IOP and 1211 DEGs for MMI, with distinct expression patterns. These patterns correlated with underlying mechanisms of action, such as deiodinase inhibition by IOP or thyroperoxidase inhibition by 6-PTU and MMI. Physiological effects, especially delayed hatching and decreased swim bladder size, are in accordance with the adverse outcome pathway concept, since impaired underlying muscle fibre development could already be observed at the gene level. The results for IOP and MMI are also supported by results for T3 and 6-PTU and demonstrate the applicability of the approach for screening purposes. Overall, our methodology demonstrated sensitivity comparable to currently utilized guideline tests that involve extensive animal use. Therefore, our experimental design, in alignment with OECD guideline 236, has potential for replacing existing guidelines in a 3R compliant manner, enhancing early hazard assessment of chemicals.

## **1.06.P Exploring Long-Term Ecological Impacts: From Epigenetic Biomarkers to Multigenerational Genomic Effects of Environmental Contaminants**

### **1.06.P-We023 Amelioration of Transgenerational Liver Disease by an Epigenetic Modifier Treatment**

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Epigenetic inheritance of environmentally and lifestyle-induced phenotypes has been demonstrated in many model organisms in a laboratory setting, suggesting that current generations could be harboring ancestral exposure-induced molecular memories, or future generations will be carrying such memories due to exposure at the current generation. Ancestral exposure-related transgenerational traits may be detrimental at the population level if occurring in a natural population. It is essential to develop strategies to correct transgenerational abnormalities before they are transferred to offspring via germline transmission or to block the heritable pathways leading to the abnormal health phenotype before they induce the disease. As a proof of concept, we investigated using medaka fish as test subject, whether the environmentally induced transgenerational non-alcoholic fatty liver disease (NAFLD) can be reversed by epigenetic modifiers and NAFLD development. Ancestral bisphenol A (BPA) exposure from the day of fertilization through 15 days after fertilization at F0 generation led to transgenerational NAFLD leading to nonalcoholic steatohepatitis (NASH) at F2 generation, which persisted for five generations. The intergenerational transmission of the disease trait was mediated by both male and female germlines. The severity of the disease was sexually dimorphic- with females affected more than males. Past F2 generation, the BPA-exposure-initiated transgenerational NAFLD started after puberty, developed in adulthood, and progressed toward NASH as the fish aged. We performed transcriptomic, metabolomic, and methylome analyses to understand the mechanisms involved. An integrated -omics analysis revealed impairments in metabolic pathways involved in proline, tryptophan, and bile acids. A larger percentage of transgenerational differentially methylated regions (DMRs) present in the NAFLD liver at the F2 generation were removed in the F4 generation during germline transfer, but the phenotype persisted with comparatively reduced severity. Treatment of the fish with a known epigenetic modifier resulted in a) correction of the majority of BPA-induced DMRs, irrespective of their methylation status, b) correction of the stressor-induced DNA methylation alterations, and c) blockade of development and progression of NAFLD in adulthood. The present results suggest that low-concentration epigenetic erasures may be useful for correcting BPA-induced transgenerational liver injury.

### **1.06.P-We024 Copper Exposure During Early Life Results in Long Term Alterations in Copper Responses Within the Life Span of Individuals and Across Generations**

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Metals are important contaminants in freshwater systems with concentrations reaching toxic levels to fish in some regions affected by mining activities, industrial or agricultural effluents. In regions affected by mining, genetically distinct populations of fish have been identified, capable of living in metal contaminated waters above the lethal thresholds for their naïve counterparts. In addition to the well described genetic adaptation to toxic metals, we hypothesised that other mechanisms could be at play and mediate rapid alterations in susceptibility to toxic metals within the life span of individuals and subsequent generations following exposure to toxic metals. To test this, we exposed stickleback embryos to a sublethal concentration of copper during embryogenesis and assessed their responsiveness to copper in the adult life stage using a series of measures including transcriptomics, DNA-methylome and microbiome analysis. We also tested the sensitivity to copper of the exposed group in comparison with naïve fish over three subsequent generations. We observed a greater tolerance to copper in the F1 and F2 generations but this effect was no longer visible in the F3 generation. At the mechanistic level, transcriptome, microbiome and DNA-methylome analysis revealed that F0 fish which had been pre-exposed to copper had a greater ability to respond to copper via adaptive mechanisms avoiding the adverse responses observed in naïve fish exposed to copper in parallel. Together these datasets provide evidence of the ability of animals to rapidly acclimate to stressors in their environment and the transient nature of those effects.

### 1.06.P-We025 Identification of Repressive Histone Methylation-mediated Reproductive Toxicity of Chemical Additives in *C. elegans* Using the Adverse Outcome Pathway (AOP) Concept and Benchmark Concentration Analysis

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Although histone methylation has been proposed as the toxic mechanism of various environmental chemicals due to its role in transcriptional regulation, relatively little is known about the hazard information and adverse outcome pathways (AOPs) for chemicals inducing these epigenetic changes. This study reports the role of repressive histone marks in toxicity to environmental chemicals and a strategy for applying epigenetic marks to an AOP framework. We measured reproduction, germline de-silencing, histone methyltransferase (HMT) activity, histone methylated protein expression, and gene expression in *Caenorhabditis elegans* exposed to environmentally pervasive chemical additives [di (2-ethylhexyl) phthalate (DEHP); hexabromocyclododecane (HBCD); tetrabromobisphenol A (TBBPA); bisphenol A (BPA); triclosan (TCS)]. In addition, benchmark concentrations (BMCs) for the experimental values with multiple concentrations were calculated to quantify the POD of each endpoint. TCS and TBBPA showed higher reproductive toxicity, increasing H3K9me3, H3K27me3, and HMTs activities in a concentration-dependent manner. These reproductive defects were alleviated following H3K27-specific HMT inhibitor (GSK343) exposure, suggesting that repressive histone methylation plays a key role in the adverse outcomes of TCS and TBBPA. The GSK343 co-treatment with the two chemicals also altered the expression of vitellogenin (*vit-1*, *vit-3*), xenobiotic metabolism (*cyp-35a2*, *gst-4*, *fmo-2*), and oxidative stress response (*ctl-2*) genes. Collectively, "HMT activation" can be proposed as an MIE that causes subsequent KEs such as H3K27 methylation and histone methylation-regulated transcriptional responses in epigenetic AOP for TCS and TBBPA. Furthermore, the utilization of BMC analysis and selective inhibitor assay can be useful to enhance the essentiality and empirical support, which are crucial considerations for AOP.

### 1.06.P-We026 Multigenerational Exposure to Antidepressants Induces Endocrine Disruption in the Estuarine Polychaete *Capitella teleta*: From Molecular- to Population-Level Effects

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Due to the continuous release of low doses of chemicals into the environment, environmental risk assessment of chemicals (ERA) is evolving in order to meet the new regulatory requirements to protect wildlife and nature. Long-term exposures and exposures across multiple generations are some of the new frontiers to assess the adverse effects of chemicals on non-target species. Current knowledge on the sublethal effects of lipophilic chemicals on sediment-dwelling invertebrates is scarce, and thus, the impacts on key ecological benthic species are still quite unexplored. This study is the first to expose three generations of the estuarine deposit-feeding polychaete *Capitella teleta* to environmentally realistic concentrations of the lipophilic antidepressant sertraline.

Selective serotonin reuptake inhibitors (SSRIs), like sertraline, have been proven to exert neurotoxic and endocrine-disruptive effects in non-target species even at low concentrations. We developed a conceptual adverse outcome (AOP) highlighting sertraline's potential mechanisms of action (MoA) underlying phenotypical changes. Therefore, herein we examined life-history traits and reproductive-related endpoints proven valuable when forecasting population impact of chemical exposure, to assess sertraline repercussions on *Capitella teleta*. Further, we analyzed the alteration of serotonin levels, energy allocation, and the population-level consequences of sediment-associated sertraline exposure. The results reveal that sediment-associated sertraline has detrimental effects on egg production, the hatching success of the progeny, and sex distribution over time and across generations. This study emphasizes the importance of conducting multigenerational studies (e.g., as part of the current ERA) to assess the adverse effects of low concentrations of chemicals in conditions simulating environmentally relevant scenarios to preserve natural ecosystems.

### 1.06.P-We027 Trans-/multi-generational Reproductive Toxicities Induced by Zearalenone Associated with Epigenetic Changes in *Caenorhabditis elegans*

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Zearalenone, a mycotoxin widely found in crops, poses an increased risk of contamination globally due to the effects of climate change. Based on this concern, there is a clear necessity to study the trans-/multi-generational toxicology of zearalenone, as environmental exposures often have long-term effects across subsequent generations. To achieve these research goals, relatively sensitive reproductive endpoints were investigated for five generations in the *Caenorhabditis elegans* model. Worms were exposed to zearalenone for 72 hours to simulate long-term exposure and produce filial generations.

The results showed that 50  $\mu\text{M}$  zearalenone exposure at parental generation can only induce transmitted reproductive defects in the first and second filial generations. As for relatively lower 0.1 and 1  $\mu\text{M}$  continuous exposure, the amplification of toxicity was observed with each successive generation, even if the initial exposure in the parent generation showed no apparent effects. The inheritance of toxicities is regulated by epigenetic changes at the molecular level to alter gene expressions without affecting sequences through chromatin modifications, such as histone methylation. Our results showed that the parental zearalenone exposure decreased expressions of genes encoding histone methyltransferases, including *set-2*, *met-2*, *mes-2*, and *mes-4*, indicating a disruption in epigenetic processes. Besides, the downregulation of gene expression exhibited a trend towards recovery in subsequent non-exposed generations. Interestingly, the trans-generational reproductive toxicities caused

by zearalenone in the second filial generation might mainly be attributed to the decline in *mes-4* expression observed across three generations.

In conclusion, these findings emphasize the significance of zearalenone-induced trans-generational toxicological effects on reproduction and linked epigenetic alterations. We hypothesize that zearalenone disrupted epigenetic regulation in the parental generation, resulting in abnormal epigenetic inheritance across generations, ultimately leading to declined transcription levels of *mes-4* until the second filial generation. Moreover, the lack of *mes-4* led to reproductive defects, contributing to trans-generational effects.

#### **1.06.P-We028 Aquatic neurotoxins – Multi-parametric Analysis of Saxitoxins Effects on *Daphnia magna***

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Saxitoxin and its derivatives are potent natural aquatic neurotoxins produced by certain cyanobacteria and marine algae species during harmful cyanobacterial and algal blooms. These harmful bloom events and the toxins produced during these events are a human and environmental health concern worldwide. Indeed, their occurrence, frequency and severity is predicted to keep increasing due to ongoing climate change scenarios. Saxitoxins constitute an interesting case study since they are produced by both freshwater and marine phytoplankton species, meaning that exposure to these toxins may occur in both aquatic compartments. Despite saxitoxins human health impacts being the focus of extensive research over the past years, adverse effects on other biota and particularly in the aquatic biota are still largely unexplored. This work aims at evaluating the effects of exposures of the model cladoceran *Daphnia magna* to an environmentally relevant concentration of saxitoxin (30 µg/L), that also corresponds to the safety guideline established by the World Health Organization (WHO) for these toxins in recreational freshwaters. Saxitoxin toxic effects were assessed through a comprehensive array of physiological (heart rate), neurotoxicity (total cholinesterases activity), biochemical (antioxidant enzymes activity and lipid peroxidation) and epigenetic biomarkers (5-mC global DNA methylation). Saxitoxin exposure resulted in decreased heart rate, total cholinesterases activity and catalase activity. Contrarily, other antioxidant enzymes, namely glutathione-S-transferases and selenium-dependent glutathione-peroxidase had their activity increased, together with lipid peroxidation levels. Global DNA 5-mC level was significantly decreased in exposed organisms. Results showed that even an ecologically relevant concentration of saxitoxin may cause changes on the antioxidants defenses, leading to oxidative stress, as well as significant physiological impairments and epigenetic alterations in *D. magna* individuals. This study highlights effects on critical molecular and cellular pathways caused by saxitoxin in *Daphnia*, suggesting that these toxins may represent a marked challenge to their thriving even at a concentration deemed safe for humans by the WHO.

#### **1.06.P-We029 EPIBOOST: A Project Aiming to Link Epigenetic Changes to Phenotypic Outcomes in Microalgae, Microcrustaceans and Fish**

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Epigenetics has been providing robust and reliable biomarkers for human health diagnosis and therapy, but this step is still largely unexplored in Ecological Risk Assessment (ERA). Many shortcomings contribute to this scenario, including the scarcity of comprehensive functional genomics information on ecotoxicological models, the still short standardization of assessment protocols, the budget required to run epigenomic studies, or, importantly, the need to establish consistent links between epigenetic modifications and downstream effects, primarily gene expression and then phenotypic impairment, allowing reliable extrapolations at larger ecological scales. This link is the focus of the EPIBOOST project, a Twinning Horizon Europe Action. The research component of the project aims to advance knowledge on global and gene-specific DNA methylation changes induced by legacy and emergent aquatic contaminants, using a metal, an antibiotic and a neurotoxin as model chemicals. Furthermore, it extends towards assessing the consequent gene expression changes and phenotypic effects, ranging from the sub-cellular to the population level, concerning specific (e.g. photosynthetic efficiency, neurotoxicity) and more general (e.g. growth rates, behavior) responses to stress. This integrated assessment framework is applied to freshwater and marine microalgae, microcrustaceans and fish, allowing comparative approaches between environmental compartments and different organisms within compartment for a given stressor. Such comprehensive framework will ultimately allow a meaningful insight on the adverse outcome pathways for the selected contaminants, and particularly support the validation of epigenetic modifications as biomarkers of relevance in supporting ERA. As a Twinning Action, EPIBOOST is also strongly dedicated to training. A large group of researchers are being trained hands-on through the research course, but many opportunities have been also created for external researchers and students through the development of Advanced Courses, Summer Schools and Workshops dedicated to transferring scientific and technical/technological knowledge in the field of environmental epigenetics.

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#### 1.06.P-We030 Multigenerational epigenetic effects of long-term exposure of *Daphnia magna* to diazinon.

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The widespread use of insecticides is leading to growing concerns about their impact on aquatic macrocrustacean. In ecotoxicology, *Daphnia magna* is used as a model for evaluating the effects of chemicals on this class of organisms. Our study focuses on the multigenerational effect of diazinon on *Daphnia magna*. Literature and our previous results highlight various effects of diazinon on *Daphnia magna* during parental exposure. These effects can be physiological modifications and behavioural alterations. In particular, we observed alterations in reproduction, growth and survival, suggesting the transmission of deleterious effects from one generation to the next. Underlying mechanisms, such as epigenetic alterations, were explored to explain the persistence of diazinon effects over several generations. In this study, we aim in identifying epigenetic markers, in particular DNA methylation, across generations of *Daphnia magna*. We set up a rigorous experimental protocol, exposing exclusively parents for a period of 7, 10 and 15 days to diazinon. The main objective was to detect the effects of parental exposure on subsequent generations, that were not directly exposed to diazinon. By focusing on DNA methylation as an epigenetic marker, we aim in understanding potential alterations in gene regulation that could be transmitted epigenetically from one generation to the next. To obtain DNA methylation, a reduced representing bisulfite sequencing (RRBS) mode was applied. We observed differences in methylation patterns between 7-day-old exposed parents (F0) to concentrations of 30 ng/L in contrast to 7-day-old control parents (F0). Notably, discernible differences were identified within the individuals of the first generation (F1). Moreover, within the F1 generation, an increase in the count of methylated CpG sites was observed. This phenomenon was particularly notable in individuals whose parental exposure extended from 7 days to 15 days with a number of methylated CpGs sites from 81 to 231 methylated CpG sites respectively. Initial results showed an increase in DNA methylation as a function of parental exposure time. Identification of the genes involved according to the localization of methylation on CpG sites and regions is currently in progress. Our results will shed light on the mechanisms underlying the multigenerational effects of diazinon.

#### 1.06.P-We031 The Hidden Dangers of Water-Soluble Polymers: Unraveling Epigenetic Changes across multiple generations of *Daphnia magna*

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Water-soluble polymers (WSPs) are widely used in many industrial, medical and consumer products due to their physical-chemical characteristics that allow their ability to dissolve in water. Despite their widespread applications, these polymers remain unregulated as either plastics or chemical contaminants leading to their continuous release into the environment. Consequently, their presence in the ecosystem can be considered ubiquitous, raising concerns about potential threats to the health of freshwater organisms, including keystone species as *Daphnia magna*. Considering the significant role of epigenetic mechanisms in environmental stress response and regulation and their potential transmission across generations, their assessment is crucial for understanding and mitigating the ecological impact of these WSPs. Thus, in this context, this project aims to assess the transgenerational inheritance of epigenetic changes (global cytosine methylation) across multiple generations in *D. magna* following exposure to two WSPs commonly used in medical applications: the Polyethylene glycol (PEG) and the Polyvinylalcohol (PVA), at the environmental concentration of 1 µg L<sup>-1</sup>. Specifically, this study focuses on the inheritance of epigenetic changes transferred to subsequent generations of *D. magna* even if the triggering contaminant is removed. This transgenerational experiment involved a 17-day exposure of the first generation (F0) to both the WSPs, followed by subsequent generations (F1, F2, and F3) exposed to the same contaminants or subjected to a recovery phase, representing the progeny returned to the fresh culture medium. Each generation underwent a 17-day exposure period after which, DNA was extracted for the analysis of the global cytosine methylation.

Our study aims to provide deeper insights into the impact of WSPs on epigenetic processes, their heritability across generations, and their correlation with organism-level effects. These findings hold broader implications for comprehending the ecological impacts of WSPs, since their transgenerational effects on a keystone species like *D. magna* can potentially disrupt population dynamics and community structures in aquatic ecosystems. Therefore, our results can contribute to better-informed environmental policies aimed at regulating the use of WSPs and minimizing their impact on aquatic environments

#### 1.06.P-We032 Assessing Multigenerational Effects of Exposure to Crude Oil on the Arctic Keystone Species *Boreogadus saida* and *Gadus morhua*, and the Model *Danio rerio*

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Understanding the impacts of petroleum exposure on reproducing fish and subsequent generations is crucial, as petroleum and its compounds may disrupt the endocrine system, leading to altered gamete quality and reproductive success. Parental exposure to petroleum can induce changes in epigenetic marks in both parents and offspring and may cause morphological deformities in unexposed offspring. Here, we explore three mechanisms of toxicity resulting from parental petroleum exposure: endocrine disruption, maternal transfer of toxic compounds, and epigenetic inheritance. Employing a comprehensive approach, we showcase preliminary experimental data and outline upcoming experimental designs that will shed light on these mechanisms.

Endocrine and epigenetic alterations along the reproductive axis are assessed on keystone arctic fish polar cod *Boreogadus saida* exposed to crude oil during late vitellogenesis. Preliminary data on the impact of exposure on gonad maturation, sperm motility analyses, plasma sex-steroid analysis and expression of key genes will be presented. Furthermore, oil-induced changes in DNA methylation transferred to first generation offspring will be analyzed through an analysis of gametes and embryos from a parentally exposed Atlantic cod *Gadus morhua*, an important commercial fish species.

Epigenetic effects in subsequent generations will be analyzed using zebrafish as a model, where reproducing adults will be exposed to a crude oil water accommodated fraction (WAF). DNA methylation, miRNA, and gene expression will be analyzed in liver and gonad tissues of F1 and F2 generations to identify changes in epigenetic control and evaluate fitness indicators.

Finally, the toxicity of maternally transferred compounds will be quantitatively and qualitatively analyzed in zebrafish embryos. Lethality and sublethal effects of a synthetic mixture mimicking the bioaccumulated fraction will be compared to those from maternal transfer and waterborne exposure. Through evaluating epigenetic and phenotypic indices transferred within and across generations, this project aims to provide insight into the impacts of petroleum exposure on reproductive success and hereditary traits of fish populations.

#### **1.06.P-We033 Reproductive Toxicity of the Cadmium Exposome: A Transgenerational Study With *C.elegans***

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The exposure and subsequent accumulation of cadmium within biological systems triggers destructive outcomes as it displaces essential metals such as zinc (Zn). Cadmium induces the synthesis of metallothioneins (MTs) and antioxidant proteins that are involved in neutralizing the toxicity. Despite the activation of these detoxification mechanisms, cadmium has the potential to exert long-lasting direct/indirect consequences of exposure that might also affect the (unexposed) next generation(s).

*Caenorhabditis elegans*, a well-established nematode organism model, was chosen to study the changes in the accumulation and the distribution of cadmium in parents and the resultant (unexposed) progeny by means of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). In addition, the transcriptomic fingerprints of the cadmium exposome was defined by RNAseq and linked with key life-cycle endpoints (reproduction and growth). In summary, whilst metals were not shown to be transferred from the exposed parents to the unexposed progeny, and no transgenerational differences were observed in terms of growth, a significantly reduced reproductive capability was apparent. In addition, the transcriptome analysis of the unexposed F1 generation identified the upregulation of numerous genes (e.g., *mtl-1*, *T22F3.11*, and *C17F4.3*) which were upon further examination by qPCR confirmed their upregulation also at the F2 generation. This suggests their possible involvement in the transmission of transgenerational effects within the context of metal stress. To conclude, cadmium toxicity has long-lasting effects that are not limited to the direct exposure of individuals, but unexposed offspring can exhibit distinct consequences, namely reproductive toxicity and the alteration of gene expression, that can last for at least two generations.

#### **1.06.P-We034 Assessing the Effects of Imazalil on Hepatic DNA Methylation and Gene Expression Profiles in *Xenopus tropicalis* Frogs**

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Amphibians are the most vulnerable group of vertebrates, with almost half of the species facing threat of extinction. The drivers are mainly anthropogenic, and include environmental pollution. Pesticide contamination in waters and soil is common in areas with intensive agricultural activity, and often occurs in tropical areas where amphibians species richness and abundance is high. Imazalil, for example, is an azole fungicide used in post-harvest treatment of fruits with reported water concentrations near plantation areas in central America reaching concentrations around 100 µg/L. *In vivo* studies evaluating effects of imazalil in vertebrates focus on rodent and fish models, and studies evaluating effects on amphibians are lacking. Reported effects include reproductive alterations, associated with its anti-androgenic mechanisms, and hepatotoxic effects, such as alterations in lipid and glucose metabolism that could lead to metabolic diseases. Therefore, the present study aims to evaluate the effects of imazalil on epigenetic and gene expression hepatic profiles in *Xenopus tropicalis* frogs. To this end, 45 days post-metamorphosis juveniles were exposed to 17 µg/L of imazalil during 15 days and DNA and RNA was extracted from liver samples. Genome-wide DNA methylation levels were analyzed by using Reduced Representation Bisulfite Sequencing (RRBS), and RNA expression levels by using RNA sequencing. Epigenetic marks such as DNA methylation can be important early predictors of organismic response to pollutants, often leading to changes in gene expression that can ultimately cause physiological long-term effects. Developmental effects of xenobiotics are often studied during early stages, however, understanding the effects during pubertal development is also important, as hepatic functions are developed during later stages. The exposure to an environmentally relevant concentration of imazalil caused alterations in the liver methylome and affected the transcriptome in pathways related to metabolism. We expect that the present study will contribute to understanding the link between epigenetic mechanisms and gene expression patterns in animals exposed to pollutants. This could help to better comprehend the connection of environmental pollution with the global amphibian decline, but also suggest possible implications to human health, due to the substantial degree of synteny between *X. tropicalis* and mammalian genomes.



## 1.06.P-We035 Exploring the Molecular Response of Anthropogenic Impacts on Feral Brown Trout Using a Translocation Experiment

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Freshwater bodies are characterised by a growing number of pollutants, resulting in the exposure of resident organisms to complex mixtures of chemicals. The effects of these exposures are difficult to assess, and impact-oriented *in situ* studies are scarce. This study aimed to explore molecular response patterns using RNAseq analyses to characterise the impacts of complex environmental exposure scenarios on feral brown trout (*Salmo trutta*) collected along a pollution gradient of a well-studied small river (Holtemme) in Central Germany. To explore the adaptability of these fish populations, we also conducted translocation experiments by relocating fish from the reference site to sites upstream and downstream of the wastewater treatment plant (WWTP) Silstedt. In comparison to fish caught and caged at the reference site (group RR), fish caught and caged at the WWTP-influenced site (group BB) exhibited three times greater number of differentially expressed genes than fish translocated to this site from the reference site (group RB). Gene ontology enrichment revealed that biological processes connected to signalling as well as catabolic and metabolic processes were commonly enriched in fish exposed downstream of the WWTP. Furthermore, differentially expressed genes in group BB were related to a variety of biological processes semantically connected to response to toxic substances, while those in group RB were primarily linked to response to oxidative stress. Finally, overrepresentation of processes connected to leukocyte regulation were only found in group RB and translational processes were enriched in group BB. Differential gene expression as well as connected biological processes differed considerably between translocated and locally adapted individuals. This is a strong indication that adaptational processes play an important role when interpreting transcriptomic response patterns.

## 1.06.PC Exploring Long-Term Ecological Impacts: From Epigenetic Biomarkers to Multigenerational Genomic Effects of Environmental Contaminants

### 1.07.A Fish Model Species in Human and Environmental Toxicology

#### 1.07.A.T-01 A Biotechnological Metabolization System has the Potential to Improve the Predictive Ability of the Fish Embryo Acute Toxicity (FET) Test with the zebrafish (*Danio rerio*)

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The Fish Embryo Acute Toxicity (FET) Test with zebrafish embryos (*Danio rerio*) was developed to assess the acute fish toxicity of chemicals or environmental samples as a replacement for the Acute Fish Test (AFT) with juvenile fish. However, the FET is not yet established in the regulatory context. One reason is the (postulated) difference between the biotransformation capacities of embryos and juvenile fish, which might lead to an under- or overestimation of toxicity.

The present study was designed to develop a procedure for external metabolization of test substances prior to testing in the FET. The workflow allows simultaneous exposure of the embryos to the maternal substances and their potential metabolites throughout the entire exposure period. After a 2 h incubation of the samples at 37 °C with a rat liver S9 homogenate or a biotechnological, animal-free (ewoS9R) metabolization system, zebrafish embryos are added shortly after fertilization and incubated up to 120 hours post-fertilization at 26 °C. Five biotransformable model substances (allyl alcohol, benzo[a]pyrene (B[a]P), chlorpyrifos (CP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and bisphenol A (BPA)) were evaluated for embryotoxicity with and without external metabolization. Non-embryotoxic concentrations of rat S9 and ewoS9R, as well as NADPH necessary for CYP activity, were determined individually and in combination. The metabolic capacities of both S9 homogenates were assessed as exemplary for the enzymes cytochrome P450 1A1, 1A2 and 1B1 *via* a fluorescence-based EROD assay.

Only for allyl alcohol, external metabolization with both rat S9 and ewoS9R resulted in significantly higher embryotoxicity than under non-premetabolized conditions and, thus, in a better correlation of FET and AFT data. For B[a]P, CP, TDCPP and BPA, there was no relevant difference between data derived from the FET (with and without pre-metabolization) and literature AFT data, even though the FET results with and without pre-metabolization differed significantly for BPA (with rat S9 and ewoS9R) and TDCPP (rat S9 only).

External pre-metabolization appears to be a promising add-on to the FET protocol to improve the correlation with AFT data of certain biotransformable substances and might help to strengthen the FET as an alternative to the AFT and finally to reduce or replace sentient animals used for acute fish toxicity data in the regulatory context.

#### **1.07.A.T-02 Assessment of the impact of realistic mixtures of plant protection products on *Danio rerio***

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Plant Protection Products (PPPs) are a current environmental problem due to their intensive use in farming systems to secure crop yields. In fact, in the European Union, 466 pesticide-active ingredients are currently approved. Due to the excessive and indiscriminate use of these substances, substantial quantities enter water systems, posing a significant risk to non-target organisms living there. To date, the risk assessment associated with the PPPs is mainly focused on single exposure ignoring the real case scenarios where multiple PPPs are applied for the crop's protection leading to the presence of mixtures in the environment. In this context, this study conducted under the framework of the EU-H2020 SPRINT project, aimed to assess the impacts of realistic mixtures of PPPs on the model fish organism *Danio rerio*. With this purpose, 11 case study sites (CSSs) were selected, comprising 10 in Europe and 1 in Argentina. The minimal environmental concentration (MEC) of PPPs was quantified for each CSS. From that, the 5 PPPs that compose each CSS mixture were then ranked based on the Risk Quotient and frequency of presence. Following the OECD test guideline 236, embryos of the zebrafish *Danio rerio* were exposed to each of the 11 mixtures at four concentrations: MEC, predicted environmental concentration (PEC), 3\*PEC and 5\*PEC, plus a control. The % of mortality, % of hatching, behavioural and Cholinesterase activity (ChE) were measured after 96h of exposure. Our findings indicate minimal effects on *D. rerio* mortality, demonstrating lower susceptibility in comparison to invertebrate and producer species that were exposed to the same mixtures in the SPRINT Project. This reduced sensitivity aligns with expectations for a higher biological organism, characterized by more intricate response systems and enhanced protection mechanisms. However, the tested PPP mixtures can modulate the zebrafish behaviour, particularly evident in terms of speed, distance and turning angle. This behavioral modulation was corroborated by the inhibition observed in the ChE, suggesting a neurotoxic effect of the tested mixtures. These results are extremely relevant as they unveil effects at concentrations presently detected in aquatic systems (MEC) as well as predicted ones (PEC). It is important to emphasize the fact that these exposures are acute (96 hours), so understand the long-term impact of these mixtures that are already present in aquatic systems becomes imperative.

#### **1.07.A.T-03 Use of *in vitro* Oocyte Maturation Assays to Predict Reproductive Capacity of Fishes**

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Oogenesis is a dynamic process by which fertilizable oocytes develop from germ cells [1]. Oogenesis can be divided into a growth phase and a maturation phase. Many endocrine disrupting chemicals impair reproduction by disrupting vitellogenesis that occurs during the growth phase. From this research, several AOPs have been developed for decreased fecundity due to disruption of vitellogenesis, including AOP 30 (direct oestrogen receptor antagonism), and AOP 25 (depression of E2 synthesis through inhibition of aromatase). During the final stage of oogenesis, oocyte maturation, maturation inducing hormone (MIH) binds to membrane progesterin receptors (mPRs) on the oocyte and signals the formation of maturation promoting factor (MPF), which promotes germinal vesicle breakdown (GVBD) and oocyte maturation, resulting in a fertilizable oocyte. Our lab has been investigating inhibition of oocyte maturation as a mechanism of decreased fecundity in three model species – zebrafish (*Danio rerio*), Japanese medaka (*Oryzias latipes*), and fathead minnow (*Pimephales promelas*). This presentation will summarize a series of studies with each model species in which we develop and use *in vitro* assays of oocyte maturation to understand the molecular mechanisms by which chemical exposures at different stages of development impact oocyte maturation, as well as the potential to use this assay as a novel tool to predict reproductive performance of fish exposed to chemical stressors. Much of the presentation will focus on our work with Japanese medaka. Using an assay previously described for zebrafish we developed an assay to assess chemical inhibition of oocyte maturation in Japanese medaka. Using this assay, we show that chemical exposure at different life stages can result in impairment of oocyte maturation leading to decreased fecundity of female fish. Potential mechanisms of chemical inhibition of oocyte maturation will also be presented.

#### **1.07.A.T-04 Sperm Quality Characterization of Male Mummichog (*Fundulus heteroclitus*) in Response to Legacy Urban Contaminants**

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Historically contaminated urban rivers harbor significant loads of legacy persistent organic pollutants within sediments that remain a significant hazard to both human and ecosystem health. Endocrine disrupting compounds are of particular concern, as they influence reproductive success and threaten the viability of native fish populations over time. Impacts to female reproductive competence, including delayed oogenesis, impaired hormone signaling, and reduced fecundity have been found

across fish taxa, but descriptions of sperm-related effects are limited. Using the mummichog (*Fundulus heteroclitus*) as an ecologically relevant model, we developed and validated a field-adaptable method for measuring aquatic sperm quality using three metrics: 1) *sperm motility*, measured by computer-assisted sperm analysis (CASA); 2) *sperm energetic capacity*, measured by a firefly-luminescence adenosine triphosphate (ATP) assay; and 3) *sperm DNA damage*, measured by Comet assay. These methods were used on sperm from field-collected *F. heteroclitus* sourced from two historically contaminated US rivers (Christina River, DE, USA; Anacostia River, MD, USA) and from a clean reference location (Wye River, MD, USA). Ten male mummichog were collected from several sites within each river and sperm cryopreserved for laboratory Comet and ATP analysis following in-field CASA video capture of sperm motility characteristics. Samples across all assays displayed consistency among replicates, sites, and time, demonstrating effective sperm extraction and cryopreservation. Motility appeared reduced in contaminated sites, though high variability among fish limited the ability to detect statistical significance. Significant differences were seen in DNA damage (via Comet assay) and in energetic capacity (via ATP assay). Samples from the PAH-laden Christina River had the most severe DNA damage, but comparatively little reduction in ATP, indicating the potential for genetically damaged sperm to swim and fertilize an egg. In contrast, samples from the Anacostia River had marked decreases in ATP along with moderate to severe DNA damage. These results emphasize the importance of multi-faceted reproductive health testing, as many adverse outcomes are likely being overlooked with current standard approaches.

#### **1.07.A.T-05 Comparison of different fish model species for the assessment of thyroid hormone system disruption**

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The current OECD testing framework for assessment of potential environmental endocrine disrupting chemicals (EDCs) covers various test guidelines that primarily use the most popular fish model species, zebrafish, fathead minnow or Japanese medaka. The choice for a species mainly depends on regulatory requirements, regional preferences and experience of laboratories. For the assessment of EDC-induced effects on sexual development and reproduction, all species are equally represented in the OECD test guidelines. However, for other endocrine modalities, this is not (yet) the case. Several EU-funded and OECD-endorsed projects are currently working on the implementation of thyroid hormone system-related endpoints into fish test guidelines for EDC assessment. This is because testing of EDCs potentially targeting the thyroid hormone system is usually performed in amphibians, even though many developmental processes and molecular markers in fish could equally be used as thyroid hormone system-related endpoints for risk assessment of chemicals. A broad data basis resulting from zebrafish experiments confirms this assumption, and, consequently, a validation of test methods at OECD level has recently been initiated. Apart from this, data from other fish species are still scarce and hinder the development of internationally applicable test guidelines. This presentation will summarize and compare the available data on thyroid hormone system disruption in different model fish species (zebrafish, Japanese medaka, fathead minnow), including less frequently used species such as rainbow trout. This will help identifying knowledge gaps might help to set impulses for new research initiatives supporting the optimization of the current testing strategy for EDCs in fish. In the fish model species session, we hope to reach a broad audience of researchers working with different fish species to initiate collaborations.

#### **1.07.B Fish Model Species in Human and Environmental Toxicology**

##### **1.07.B.T-01 Adverse Effects on Eye Development of Zebrafish Embryos after Exposure to Tricyclic Antidepressants**

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Due to their presence in aquatic ecosystems and their high efficacy towards very specific molecular targets, active pharmaceutical ingredients are considered contaminants of emerging concern. Tricyclic antidepressants (TCAs) are a group of APIs that target the reuptake of neurotransmitters in the presynaptic nerve endings. Previous ecotoxicological studies with zebrafish (*Danio rerio*) revealed their potential to cause behavioural effects. However, it is unknown if effects to vision play a role in these effects.

This study aims to assess, if exposure to TCAs has effects on the development of the vertebrate eye using the zebrafish as a model organism. Effects at different levels of biological organization were measured: transcriptomics, which was assessed via mRNA sequencing at 48, 72 and 96 hours post fertilization (hpf), eye structure, which was assessed via histopathology at 120 hpf, and eye function, which was assessed via the optokinetic response (OKR) assay at 120 hpf.

Embryos were exposed to amitriptyline (AMI) and nortriptyline (NOR) in a concentration range from 30 ng/L to 300 µg/L (nominal concentrations).

OKR assay showed a significant decrease in the frequency of eye movements for AMI in concentrations of 3 and 300 µg/L, and for NOR in concentrations of 300 µg/L. Histopathology revealed the retinal pigment epithelium thickness to be

significantly increased in the 300 µg/L treatment group of AMI by 15.8% and in NOR by 15.3%. mRNA sequencing results showed effects on expression of multiple genes related to eye development. Next to eye development, gene expression effects were also found in genes related to synaptic signalling, confirming the mechanism of action to be conserved in zebrafish embryos, muscle development, neuronal development, lipid metabolism, and thyroid signalling.

As thyroid disruption can cause adverse effects on eye development, and the transcriptomics data also implies thyroid disruption to be affected, current experiments investigate the involvement of thyroid disruption in the adverse outcome pathway through which TCAs affected the eye.

The present findings provide evidence of adverse eye development as a previously unknown effect of antidepressant exposure to vertebrates on multiple levels of biological organization and contribute towards establishing an understanding for the mechanism through which this effect occurs.

### **1.07.B.T-02 How to Assess Thyroid Hormone System Disruption in Zebrafish: A Case Study with Methimazole** *Sina Volz<sup>1</sup>, Karin Lund Kinnberg<sup>1</sup>, Katrine Kirkegaard<sup>2</sup>, Pernille Raun Klausen<sup>1</sup>, Bente Frost Holbech<sup>1</sup> and Henrik Holbech<sup>1</sup>, (1)University of Southern Denmark, Denmark, (2)Biology, University of Southern Denmark (SDU), Odense, Denmark*

The thyroid hormone system (THS), a crucial regulator of vertebrate development, is a major target for a variety of endocrine-disrupting chemicals. Currently, the assessment of THS disruption in non-mammalian toxicology primarily relies on amphibian assays, whereas effects on other endocrine modalities are tested in fish studies. Given the high degree of conservation of the THS across vertebrate classes, it is likely that incorporating newly identified THS-sensitive endpoints into established OECD fish test guidelines would allow for the assessment of THS disruption in fish. The present case study thus aimed at determining suitable endpoints and developmental stages to assess THS disruption in developing zebrafish. Methimazole, a medication used to treat hyperthyroidism, was selected as a test substance due to its well-described effects on thyroid hormone synthesis.

Zebrafish were exposed to 3.2-100 mg/L methimazole, from 2 hours to 33 days post fertilization (dpf). At 5, 21, and 33 dpf, samples were taken to assess various THS-sensitive endpoints. At 5 dpf, THS disruption could be detected by gene expression analysis (altered expression of *thyroperoxidase*) and immunohistochemical assessment of intrafollicular thyroid hormone levels. Histopathological analyses of the eyes are still ongoing and will be finished by the end of the year. Following 21 days of exposure, inflation of the anterior chamber of the swim bladder was impaired; this is a known effect of THS-disrupting chemicals. At 33 dpf, THS disruption was evident on the gene expression level (altered expression of *thyroperoxidase*) and morphological level (increased height of thyroid follicular epithelium). Furthermore, effects on eye development, which is dependent on the THS, were detected via gene expression analysis and histopathology. Targeted thyroid hormone determination at 5, 21, and 33 dpf are still pending but expected to be ready for presentation in May.

Taken together, THS disruption caused by methimazole could be detected at both 5 dpf and 33 dpf. These findings indicate that THS disruption could be assessed in the fish embryo acute toxicity test (OECD TG 236) as well as the fish early life stage test (OECD TG 210). While the results of this case study are promising, it is essential to compare both specificity and sensitivity between amphibians and fish test systems to conclude whether fish studies could replace amphibian assays in the risk and hazard assessment of THS disruptors.

### **1.07.B.T-03 Early life stage toxicity of radium (<sup>226</sup>Ra) to three fish species of ecological, cultural, and commercial importance in Canada**

*Edgar Ramiro R Perez<sup>1</sup>, Charlotte Lacroix-Durand<sup>1</sup>, Karsten Liber<sup>2</sup> and David M. Janz<sup>3</sup>, (1)Toxicology, University of Saskatchewan, Canada, (2)School of Environment and Sustainability, University of Saskatchewan, Canada, (3)Western College of Veterinary Medicine and Toxicology Centre, University of Saskatchewan, Canada*

In Canada, the mining, petroleum, and metal processing industries generate waste that contain metals and radionuclides of concern, like Radium (<sup>226</sup>Ra). Despite strict discharge regulations for <sup>226</sup>Ra in effluents, a Canadian federal water quality guideline that protects aquatic organisms from <sup>226</sup>Ra exposure is not in place, casting doubt on the condition and well-being of current fish stocks. To assess the most sensitive stages of fish development (i.e., fertilization to swimup), we exposed rainbow trout (*Oncorhynchus mykiss*), white sucker (*Catostomus commersonii*), and fathead minnow (*Pimephales promelas*) to a series of environmentally relevant <sup>226</sup>Ra activities (0, 0.1, 0.5, 2.5, 12.5, or 62.5 Bq/L) using a modified version of EPS 1/RM/28 for *O. mykiss* and *C. commersonii*, and OECD method 210 for *P. promelas*. Under static renewal conditions, *P. promelas* embryos were exposed to <sup>226</sup>Ra in reconstituted hard water (160 – 180 mg/L CaCO<sub>3</sub>), while *O. mykiss* and *C. commersonii* embryos in very soft water (10 – 13 mg/L as CaCO<sub>3</sub>), with the aim of identifying sensitive toxicity endpoints. Early life stage toxicity data for *O. mykiss* and *C. commersonii* are presented, while the *P. promelas* experiment is in progress. The results indicate that <sup>226</sup>Ra was not toxic to *O. mykiss* or *C. commersonii* survival or time to stage for eyed, hatch, or swim-up. However, for *O. mykiss* only, significant increases (p<0.05) in length, mass, and the scaled mass index (SMI) were observed at lower activities of 0.1 and 0.5 Bq/L <sup>226</sup>Ra as the exposure time increased, generating a significant interaction between activities and time to stage in degree days. A deformity analysis on *O. mykiss* revealed a biologically significant increase in total frequency of deformities at 62.5 Bq/L (p=0.07). In summary, the no observed effect concentration (NOEC) for time to stage and survival endpoints was 62.5 Bq/L for both *O. mykiss* and *C. commersonii*, whereas for *O. mykiss*, the lowest observed effect concentration (LOEC) for length was 0.1 Bq/L, mass and SMI 0.5 Bq/L, and total deformity frequency 62.5 Bq/L. Other pending analyses for *O. mykiss* include targeted transcriptomics and bioaccumulation in embryos and fry, while data for *C.*

*commersonii* and *P. promelas* are pending completion. As of now, the monthly mean discharge limit of 0.37 Bq/L total <sup>226</sup>Ra in effluents may pose a potential risk to fishes in Canadian aquatic ecosystems.

### **1.07.B.T-04 2,2',4,4'-Tetrabromodiphenyl Ether Causes Depression-like Effects in Zebrafish Larvae via a Non-image-forming Visual Mechanism**

*Miao Cao, Ting Xu and Daqiang Yin, Tongji University, China*

Background: Depression is a high-incidence mood disorder that is frequently accompanied by sleep disturbances. Circadian disruption triggered from disordered non-image-forming (NIF) visual pathway is accordingly proposed as a new pathogenic factor of depression. Polybrominated diphenyl ethers (PBDEs), with visual impairment being the emerging toxicological feature, have been extensively used as flame retardants and new cohort studies reported the relationships between PBDEs and depressive symptoms.

Research Purpose: This study aimed to delineate the performances of NIF system under 2,2',4,4'-Tetrabromodiphenyl Ether (BDE-47) exposure, especially with day-night differences, and to verify the action mode of melanopsin, which is the major photopigment of NIF system, in facilitating depression-like effects in zebrafish larvae.

Methods: We employed diurnal zebrafish model for this study. The nominal BDE-47 concentrations herein were designed as 0, 2.5 µg/L, and 25 µg/L with 0.01% DMSO based on the reported environmental levels. Considering the possible involvement of the circadian system, samples were collected at various circadian time points in subsequent experiments of quantitative Real-Time PCR, immunofluorescent staining, enzyme-linked immunosorbent assay, *in situ* hybridization (ISH), etc.

Results: BDE-47 caused a distinct day-night difference in the melanopsin and associated NIF functions at 5 and 6 dpf zebrafish larvae. The differences were transmitted to clock genes and neuropeptides in SCN and impacted the adjacent 5-HT and melatonin receptors. However, the changes in the vital factors of depression and sleep were unidirectional, which led to the depression-like (including thigmotactic and sleepy) effects in BDE-47-exposed larvae. The melanopsin antagonism suggested it was possibly primarily due to the direct photoentrainment function of melanopsin.

Conclusion: BDE-47 exposure disrupted NIF visual pathway and resulted in depression-like effects, which may further exert profound health effects like mood disorders.

Implication: Our study provided mechanistic cues for the epidemiological correlation between human depression and PBDE exposure. Such mechanism successfully explained the circadian differences in some depression indicators. Of note, the neurotoxic sensitivity of melanopsin at environmental-relevant BDE-47 concentrations might indicate its implication potential as a novel target in ecological risk assessment of aquatic PBDE exposure.

### **1.07.P-Th020 Towards Standardization of the Dark/Light Transition Test with Zebrafish (*Danio rerio*) embryos. Reporting of Results from an Inter-laboratory Ring Test**

*Riccardo Massei, Dr<sup>1</sup>, Maria Christou<sup>2</sup> and Adam Lillicrap<sup>3</sup>, (1)Helmholtz Centre for Environmental Research (UFZ), Germany, (2)Norwegian Institute for Water Research (NIVA), Norway, (3)Norwegian Institute for Water Research (NIVA), Oslo, Norway*

Over the last few decades, there has been a rapid increase in the analysis of behavioral responses in zebrafish embryos following exposure to chemicals, primarily due to their potential for providing more informative data compared to traditional endpoints described in the Fish Embryo Acute Toxicity Test (OECD TG 236). One of the most commonly utilized behavioral assays is the dark/light transition test (DLTT), which involves recording and quantifying the swimming activities of hatched zebrafish eleutheroembryos under different light conditions. Commercially available behavioral systems are used to conduct this test. However, the DLTT lacks standardization, and guidelines have yet to be established. As a result, zebrafish laboratories employ in-house protocols to record the activity of zebrafish eleutheroembryos. Additionally, different statistical tests are employed to explain the results and analyze the output. Consequently, comparing the results across different studies becomes a challenging task and poses a potential obstacle to bring these tests to regulators. Prior to developing an internationally accepted standard test guideline, it is necessary to characterize and understand the general variation in the DLTT. Currently, it remains unclear whether the variation could be attributed to the use of different standard operating procedures (SOP) or other variables such as exposure conditions, data analyses or even the strain of the fish. Previous attempts by labs to conduct different ring trials were hindered by non-standardized exposure scenarios and a low number of participants, making interpretation of results difficult. To address these limitations, a ring test involving 24 institutions from both the European Union (EU) and non-EU countries was organized in 2023 and an SOP was distributed to all the participants. Participants exposed zebrafish embryos to either caffeine, a known neurotoxicant, or 3,4-dichloroaniline from 3 to 120 hours post fertilisation (hpf) and performed a DLTT at 96 and 120 hpf. The primary goals of this ring test are to: 1) determine the reproducibility of the DLTT with zebrafish embryos across different laboratories; 2) understand the potential experimental factors and test procedure key steps that contribute to observed variation in the general DLTT; 3) produce a comprehensive dataset that can be utilized to test different statistical approaches and 4) develop user-friendly data analysis pipeline for statistical analysis.

### **1.07.P-Th021 Bisphenols A alternatives alters Thyroid Hormone System (THS) sensitive endpoints in zebrafish - are they safer alternatives?**

*Pernille Ambus Hansen<sup>1</sup>, Jane Ebsen Morthorst<sup>2</sup> and Henrik Holbech<sup>2</sup>, (1)Biology, University of Southern Denmark, Denmark, (2)University of Southern Denmark, Denmark*

Since REACH included Bisphenol A (BPA) under Category 1B due to toxicity for reproduction and endocrine disruption properties various BPA-alternatives like BPE, BPAP and BPS-MAE, have been marketed. We investigate if these BPA-alternatives affects the thyroid hormone system (THS), as have been shown for BPA, or if they are safer alternatives in relation to THS disruption. We do that by proposing an Adverse Outcome Pathway (AOP) in zebrafish embryos <120 hours post fertilization (hpf), to investigate THS sensitive endpoints. The experimental design is based on the OECD Test Guideline 236: Fish Embryo Toxicity Test (FET) with addition of the THS sensitive endpoint posterior swimbladder inflation and analysis of swimming performance at the end of the test.

We tested BPE, BPAP and BPS-MAE. As an example, for the Bisphenol E (BPE), we exposed zebrafish larvae from 2 hpf – 120 hpf in a semi-static renewal system to 5 concentrations (5 µg/L, 25 µg/L, 125 µg/L, 625 µg/L and 3125 µg/L). BPS-MAE and BPAP were tested with a similar approach at different concentrations.

In the test for BPE we found a decreased swimbladder inflation rate and a change in the swimming performance which can be regarded as an adverse effect with population relevance. This indicates that BPE adversely affects zebrafish embryos via disruption of the thyroid hormone system. We will present the full datasets from these tests including internal T3- and T4 measurements, statistical analysis of the swimbladder inflation rates and a potential correlation between decrease in swimbladder inflation rate and swimming performance.

We will also present if the same tendency applies for other BPA-alternatives like BPAP and BPS-MAE.

### **1.07.P-Th022 Identification of ingredients in Air Freshener Commercial Products Causing Neurotoxicity to zebrafish**

*han-Seul Lee and Kitae Kim, Seoul National University of Science and Technology, Korea, Republic of (South)*

(Introduction)Household products contain a variety of chemicals for their function. Given that we use a variety of household products on a daily basis, it is reasonable to expect that chemical exposures from the use of household chemicals contribute significantly to our overall chemical exposure. Recently, a variety of toxicities such as skin, respiratory, neurological, and systemic disorders from chemicals in these household chemicals have been reported. However, despite the presence of multiple active ingredients in a single household chemical, toxicity is often reported by listing only the toxicity caused by individual substances. It is not known which substances cause toxicity based on the active ingredients in household chemicals, or how to find them.

(Materials and Methods)Zebrafish embryos were used to evaluate the toxicity of air freshener products from a neurotoxicity perspective based on whole extract. After evaluating the toxicity of air freshener products, we sought to identify the causative agents responsible for the toxicity. To identify the neurotoxicants, we searched the literature for keywords related to neurotoxicity and investigated lipid affinities to narrow the three individual substances. Among the three identified causative agents, the one with the highest initial toxicity in zebrafish embryos was validated by analyzing neurobehavioral changes after long-term exposure to adult zebrafish. Neurochemicals were analyzed in the brains of adult zebrafish to determine the mechanism of neurotoxicity.

(Conclusions)Toxicity tests and behavioral assays using zebrafish embryos indicated that whole and extracted mixtures of the air freshener product could be neurotoxic. To identify the neurotoxicants, we searched the literature for keywords related to neurotoxicity and investigated lipid affinities to narrow the list, and citronellol, geraniol, and linalool were identified as suspected toxicants. The neurobehavioral changes we observed after long-term exposure of adult zebrafish to citronellol, geraniol, and a mixture of both substances demonstrated that the individual substances we identified were neurotoxic. Our research represents the pioneering effort in formulating a methodology for identifying toxic culprits in household products. Moreover, we have substantiated the toxicity of these identified agents utilizing the zebrafish animal model. Moving forward, we plan to extend this work to a broader range of product groups in a follow-up study.

### **1.07.P Fish Model Species in Human and Environmental Toxicology**

#### **1.07.P-Th020 Towards Standardization of the Dark/Light Transition Test with Zebrafish (*Danio rerio*) embryos. Reporting of Results from an Inter-laboratory Ring Test**

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this test. However, the DLTT lacks standardization, and guidelines have yet to be established. As a result, zebrafish laboratories employ in-house protocols to record the activity of zebrafish eleutheroembryos. Additionally, different statistical tests are employed to explain the results and analyze the output. Consequently, comparing the results across different studies becomes a challenging task and poses a potential obstacle to bring these tests to regulators. Prior to developing an internationally accepted standard test guideline, it is necessary to characterize and understand the general variation in the DLTT. Currently, it remains unclear whether the variation could be attributed to the use of different standard operating procedures (SOP) or other variables such as exposure conditions, data analyses or even the strain of the fish. Previous attempts by labs to conduct different ring trials were hindered by non-standardized exposure scenarios and a low number of participants, making interpretation of results difficult. To address these limitations, a ring test involving 24 institutions from both the European Union (EU) and non-EU countries was organized in 2023 and an SOP was distributed to all the participants. Participants exposed zebrafish embryos to either caffeine, a known neurotoxicant, or 3,4-dichloroaniline from 3 to 120 hours post fertilisation (hpf) and performed a DLTT at 96 and 120 hpf. The primary goals of this ring test are to: 1) determine the reproducibility of the DLTT with zebrafish embryos across different laboratories; 2) understand the potential experimental factors and test procedure key steps that contribute to observed variation in the general DLTT; 3) produce a comprehensive dataset that can be utilized to test different statistical approaches and 4) develop user-friendly data analysis pipeline for statistical analysis.

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*han-Seul Lee and Kitae Kim, Seoul National University of Science and Technology, Korea, Republic of (South)*

(Introduction)Household products contain a variety of chemicals for their function. Given that we use a variety of household products on a daily basis, it is reasonable to expect that chemical exposures from the use of household chemicals contribute significantly to our overall chemical exposure. Recently, a variety of toxicities such as skin, respiratory, neurological, and systemic disorders from chemicals in these household chemicals have been reported. However, despite the presence of multiple active ingredients in a single household chemical, toxicity is often reported by listing only the toxicity caused by individual substances. It is not known which substances cause toxicity based on the active ingredients in household chemicals, or how to find them.

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geraniol, and a mixture of both substances demonstrated that the individual substances we identified were neurotoxic. Our research represents the pioneering effort in formulating a methodology for identifying toxic culprits in household products. Moreover, we have substantiated the toxicity of these identified agents utilizing the zebrafish animal model. Moving forward, we plan to extend this work to a broader range of product groups in a follow-up study.

#### **1.07.P-Th023 A Comparative Study of toxic pathway according to the Large Microplastic Shape Characteristics (Polyethylene Terephthalate) on juvenile Rockfish *Sebastes schlegelii***

**Kwang-Min Choi<sup>1</sup>, Seong Hee Mun<sup>2</sup>, Dongju Shin<sup>1</sup> and Jee Hyun Jung<sup>1</sup>,** (1)Korea Institute of Ocean Science and Technology (KIOST), Korea, Republic of (South), (2)Korea Institute of Ocean Science and Technology (KIOST), Yeongdo-gu, Korea, Republic of

Microplastics are heterogeneous materials which diverse (not only in particle size and shape, but also in polymers types depends on the chemical composition including the additives products). It still remains unknown what the plastic characterization itself are the major factor and impacts for microplastic toxicity. In the study, two different shape types (fragment and fiber type) microplastics that dominate the coastal environmental types were administered orally in juvenile. In this study, LMP was made of polyethylene terephthalate (PET) in the form of fragments and fibers with a size of 3 mm and administered orally using a gelatin capsule. After exposure, fiber-type PET-LMP (FiPET-LMP) induced stronger cytotoxicity than fragment-type PET-LMP (FrPET-LMP), decreased antioxidant enzyme activity in hepatocytes, and increased reactive oxygen species (ROS) production in a concentration-dependent manner. Transcriptomic analysis confirmed that exposure to FrPET-LMP and FiPET-LMP affected biological processes such as energy metabolism and cell homeostasis, respectively. Based on metabolomics analysis, we found the impact of nicotinate and nicotinamide metabolism pathway involved in energy metabolism under stress caused by FrPET-LMP feeding. After FiPET-LMP ingestion, more liver metabolic pathways were affected, confirming problems with the tryptophan metabolism pathway related to inflammation. Our results can be used as reference data to evaluate the effects of PET exposure and can be useful for identifying correlations between toxic effects and physiological functional properties of marine fish after exposure to MPs.

#### **1.07.P-Th024 A Method for the Detection of Steroid Hormones in Fish Holding Tank Water**

**Emily Kennedy<sup>1</sup>, David M. Janz<sup>2</sup> and Markus Hecker<sup>3</sup>,** (1)Toxicology, University of Saskatchewan, Canada, (2)Western College of Veterinary Medicine and Toxicology Centre, University of Saskatchewan, Canada, (3)Toxicology Centre and School of the Environment and Sustainability, University of Saskatchewan, Canada

Endocrine disruption, caused by the presence of chemical compounds in the environment, poses a significant threat to aquatic ecosystems and the health of fish populations. These impacts are often directed towards steroidogenic pathways, making the assessment of circulating steroid hormone concentrations a valuable method for understanding such effects. While blood sampling is the conventional approach for steroid hormone quantification, it presents challenges, particularly in the case of small fish species like the fathead minnow commonly used in studying endocrine disrupting compounds (EDCs). Often, these fish are too small to provide sufficient blood volume for sample analyses necessitating the pooling of multiple samples and reducing statistical power. Additionally, ethical concerns arise regarding the sacrifice of a large number of animals in both laboratory and field-based biomonitoring programs for the purpose of blood collection. As such, the objective of this project was to develop a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of steroid hormones excreted into holding tank water to be used in the screening of EDCs. Using 500 ml of water collected from a tank containing 4 L of water and a single fathead minnow detection limits for 7 steroid hormones including 11-ketotestosterone and 17- $\beta$ -estradiol ranged from 0.02-0.15 ng/L. Moving forward, this method will now be calibrated by comparing concentrations of hormones in water to those in blood as well as tested for its ability to detect endocrine disruption following exposure to known EDCs. Once fully validated this method will be invaluable to the non-lethal screening of EDCs, and thus, the assessment of fish health and improving fish welfare.

#### **1.07.P-Th025 Assessing the Impact of Combined Anticancer Drugs On the Early Life Stages of *Danio rerio***

**Daniel Alexandre Bruno<sup>1</sup>, Diana Isabel Oliveira Carneiro<sup>2</sup>, Susana Loureiro<sup>3</sup> and Maria D Pavlaki<sup>2</sup>,** (1)University of Aveiro, Portugal, (2)University of Aveiro & Centre for Environmental and Marine Studies (CESAM), Portugal, (3)CESAM - Centre for Environmental and Marine Studies & Department of Biology, University of Aveiro, Portugal

Across the world, millions of patients are undergoing chemotherapy, resulting in an increased consumption of anticancer agents (AAs). After AAs are administered to patients, they can end up in the environment due to wastewater treatment plants' lack of efficacy. Furthermore, in real-world scenarios, AAs do not exist isolated in the environment but rather in mixtures with other AAs and/or contaminants. Therefore, it is urgent to evaluate the effects of such a potential mixture on aquatic species. In this study, we assessed the single and joint eco- and genotoxicity of three AAs, trabectedin (TRAB), doxorubicin (DOX), and oxaliplatin (OXA), commonly used in cancer treatment patients jointly or in sequence, thus increasing their possibility to be found as part of mixtures. Embryos of *Danio rerio* were exposed to the three AAs individually and in combination following the OECD 236 Fish Embryo Acute Toxicity Test to assess acute toxicity, developmental alterations and DNA damage of zebrafish embryonic stages. A full factorial design was used to assess the joint toxicity effects of the three AAs and deviations from the reference models (Concentration Addition and Independent Action) whenever possible, along with calculating synergistic ratios. Using no-effect concentrations, the comet assay evaluated DNA damage after 96 hours of exposure to single and binary mixtures. The single acute toxicity tests revealed that TRAB and DOX caused mortality and malformations in zebrafish embryos. On the other hand, OXA did not cause any effects at concentrations up to 100 mg/L. Trabectedin was shown to be the most toxic, followed by DOX and then OXA, in terms of mortality, embryo/larvae malformations and DNA



damage. Results from the mixtures suggest a Dose Ratio Deviation between TRAB and DOX, when evaluating mortality, showing synergism when TRAB concentrations are high, and antagonism when DOX concentrations are high, while in DOX – OXA and TRAB – OXA mixtures was observed an increasing pattern of effects on zebrafish larvae with increasing oxaliplatin concentrations. Effects of the binary combinations of the three AAs show potentiation of effects in two of the mixtures, while a possible antagonism appears to occur in the TRAB-OXA mixture when larvae are exposed to lower TRAB and higher OXA concentrations. The results of this study highlight the importance of assessing the effects of AAs mixtures in the environment rather than solely focusing on their individual effects.

#### **1.07.P-Th026 Cardio- and Neurotoxicological Characterization of Sediment Flood Samples Taken After the 2021 Extreme Flood Event in Western Germany using Zebrafish Embryos**

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In July 2021, severe thunderstorms caused extreme flood events in several European countries. In Western Germany, which was one of the most affected areas, the floods resulted not only in many fatalities and infrastructural damage but also seriously affected the environment. On this background, sediment samples were taken within 5 days after the flood event around the city of Stolberg, North Rhine-Westphalia (Germany), which has a long history of metalworking and chemical industries. As part of a comprehensive ecotoxicological profiling, the sediments were tested for their cardio- and neurotoxic potential in *Danio rerio* embryos up to 120 hours post fertilization (hpf). After freeze-drying, the sediments were extracted using pressurized liquid extraction, completely dried and reconstituted with dimethyl sulfoxide for further testing. The Fish Embryo Acute Toxicity test was performed (OECD 236 and ISO 15088), with minor adjustments) to evaluate lethal and sublethal morphological endpoints of the different samples. From the concentration-response relationship, EC<sub>5</sub> and EC<sub>10</sub> were obtained for further testing. A battery of behavioural assays (spontaneous tail coiling (24 hpf), touch-evoked response (72 hpf) and light/dark transition (120 hpf)), as well as Acetylcholine esterase (AChE) activity measurement in zebrafish larvae homogenates, was conducted to assess the neurotoxicity of the samples. For evaluation of the cardiotoxic potential, sublethal morphological effects and heartbeat were analyzed, and 7-ethoxyresorufin-O-deethylase activity (EROD) was measured in zebrafish larvae homogenates. In contrast to our expectations, the neurotoxicological profiling did not reveal significant deviations from the controls, only AChE activity was slightly elevated for a few samples. For cardiotoxic profiling, however, significant effects could be observed. Increased heartbeat was detected in several samples and all samples showed significant induction of EROD activity. These results indicate a heavy pollution of the sediments with polycyclic aromatic hydrocarbons, polychlorinated biphenyls and dioxin-like compounds, which may originate from leaked fuels and heating oils as well as the remobilization and redistribution of contaminated sediments. Combined with the pending chemical analysis, the results will help to identify the actual sources of contaminants and thus contribute to a more detailed assessment of the environmental threats caused by extreme events like this.

#### **1.07.P-Th027 Characterisation of Zebrafish Embryos Developmental Stages using Automated Computer Device**

Sizenando Nogueira Abreu<sup>1,2</sup>, Ines Domingues<sup>3</sup>, Bruna Veira<sup>3</sup>, Fatima Jesus<sup>3</sup>, Amadeu M.V.M. Soares<sup>3</sup>, Rui Martins<sup>2,4</sup> and Miguel Oliveira e Silva<sup>2,4</sup>, (1)CESAM & Department of Biology, University of Aveiro, Portugal, (2)TekenBio, Portugal, (3)CESAM - Department of Biology, University of Aveiro, Portugal, (4)leeta - Deti, University of Aveiro, Portugal

Zebrafish (*Danio rerio*) embryos are a popular model increasingly used in ecotoxicology assays not only due to their characteristics (e.g., transparency and quick development) but also because they comply with the 3 R's policy promoted by the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes, being considered an alternative model for animal experimentation. Setting up a zebrafish embryo assay involve the laborious and time-consuming task of counting and selecting large number of newly fertilised embryos. The equipment for automated detection of small organisms that has been, in previous works, tested and validated for counting zebrafish eggs/embryos. In this work, the equipment was used to obtain an optic characterisation of the different developmental stages of zebrafish embryos from 2 to 7 hours post fertilisation as well of the non-viable and dead (coagulated) embryos that can always be observed together in variable degrees in the eggs' batches. Optic characterisation and direct embryo observation were performed for calibration of the computer device. Embryos showed different levels of light absorbance, having non-viable embryos lower absorbances and coagulated eggs having the higher values. Although, non-viable and viable embryos absorbances distribution show some overlap areas in the first 3 hours, data indicate that at 4-5h post fertilisation, a differentiation is observed, being the most accurate time point post fertilisation where well-developed eggs can be better differentiated from non-viable or dead embryos.

Overall data can be used to evaluate the non-viable, viable and dead embryos distribution in the eggs' batches, and it could be used as a tool to infer the levels of fertilisation success in zebrafish facilities.

#### **1.07.P-Th028 Characterising the Functional Homology of Central Nervous System Drug Targets in Fish.**

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Many pharmaceuticals designed to target receptors in the human central nervous system (CNS) are increasingly detected in the aquatic environment. Critically, the genes encoding the molecular targets of many of these drugs are highly conserved between humans (the intended target species) and fish. Consequently, it has been proposed that many are likely to be biologically active

in fish when they enter aquatic environments. However, there is limited information on whether many of these targets exhibit functional homology between humans and fish. Here we sought to address this knowledge gap for a range of CNS targets applying a suite of techniques to assess whether their gene sequence homology confers conservation of biological (pharmacological) function. Firstly, we employed bioinformatics to identify the CNS drug targets with the highest degree of similarity between humans and zebrafish (*Danio rerio*, our chosen study fish species). We then used this information to prioritise CNS drugs that also are detected widely in surface waters. We then assessed the impact of exposure to pharmacologically (from human therapeutic data) and environmentally relevant levels of these drugs on zebrafish embryo - larval development that included assessing for CNS-specific impacts (via behaviours). Bioavailability of the drugs in the embryo-larvae was also assessed using liquid chromatography with tandem mass spectrometry. Collectively, these data guided studies for more specific CNS-focussed tests designed to better establish the neurobehavioral effects of these compounds. For this, larval zebrafish with a pan neuronal genetically-encoded  $Ca^{2+}$  sensor in combination with light sheet microscopy was used to measure brain activity after exposure to each test drug for 4 days. This approach allowed us to assess the impact of drug exposure across multiple brain regions, which we then correlated with the distribution of the drug molecular targets in the brain, indicating whether orthologous targets are being modulated within the fish. Mapping brain regions affected can furthermore provide insights into likely behavioural impacts, such as those mediated by olfactory, visual or locomotory activity. A key overall aim of this work is to assess the degree of functional homology of drug targets between mammals and fish, thereby identifying more sensitive endpoints for risk assessment as well as gaining a better understanding of likely effects of these neuroactive drugs on fish in the wild.

#### **1.07.P-Th029 Combined effects of benzisothiazolinone and propyl paraben using embryonic and adult zebrafish** *Cha Eun Park and Kyunghye Ji, Yong In University, Korea, Republic of (South)*

Benzisothiazolinone (BIT) and propyl paraben (PP) are often mixed and used as preservatives in cleaning products. In the present study, the toxicity of the binary mixture to BIT and PP through *in vivo* zebrafish experiments, considering the content ratio and chemical combination of the cleaning products. Zebrafish embryos were exposed to BIT (2.29 and 229  $\mu\text{g/L}$ ), PP (0.64 and 64.5  $\mu\text{g/L}$ ), and mixtures for 96 h, and the effects on the growth hormone (GH), insulin-like growth factor-1 (IGF-1), and the transcription of genes related to GH/IGFs axis were investigated. Zebrafish pairs were also exposed to BIT, PP, and mixtures for 21 d, and the effects on sex hormones and transcription of genes related to hypothalamus-pituitary-gonad axis and vitellogenin were assessed. The mixtures had greater effects on development, reproduction, hormones, and gene expressions than individual exposure. Larvae exposed to 229  $\mu\text{g/L}$  BIT or mixtures showed significant reductions in growth. Reduced contents of GH and IGF-1 are well supported by regulation of genes associated to GH/IGFs axis. In larvae exposed to BIT, PP, or mixtures, reactive oxygen species and the levels of superoxide dismutase, catalase and glutathione peroxidase were significantly increased. Although no significant changes were observed in *vitellogenin* gene transcription, a decrease in gonadosomatic index in male fish and number of eggs were observed in mixed exposure. The results of a significant decrease in testosterone in males and a significant decrease in  $17\beta$ -estradiol in females exposed to binary mixture suggest the anti-estrogenic and anti-androgenic potential. The results of this study indicate that the toxicity of mixtures of preservatives in consumer products may be greater than the toxicity of individual substances, which may be helpful in managing the risks of mixtures in products.

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Keywords: Benzisothiazolinone; GH/IGFs axis; HPG axis; Propyl paraben; Zebrafish

#### **1.07.P-Th030 Development of toxicity test protocols for Atlantic cod (*Gadus morhua*) – a relevant test species for the Northern Atlantic Ocean**

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Atlantic cod (*Gadus morhua*) is a keystone species in the North Atlantic ecosystem, playing a crucial role in maintaining the balance of marine food webs. Additionally, it has been a vital resource for centuries, supporting fisheries that have shaped the cultural and economic basis for coastal communities. Climatic- or pollution-related impacts on cod recruitment would have devastating biological, societal, and economic consequences. We have developed a standardized protocol for ecotoxicological testing using this species based on the OECD Test No. 236: Fish Embryo Acute Toxicity (FET) which is based on the use of zebrafish (*Danio rerio*) embryos. Atlantic cod eggs are comparable to zebrafish eggs in size, but in contrast, cod eggs have a lower density (pelagic eggs), develop at lower temperature and over a longer period from fertilization to hatch (approx. 100 degree days,  $d^\circ$ ). Compared to the OECD guideline, we used larger exposure volumes ( $>100$  mL), an increased number of individuals (approximately 100 eggs per 100 mL beaker) and a lower temperature ( $8.5 \pm 1$   $^\circ\text{C}$ ). In addition, we standardized sublethal toxicity endpoints relating to cardiotoxicity and morphological deformations using automated analyses of videos and images. A positive control experiment using 3,4-dichloroaniline (DCA) revealed that cod embryos were significantly more sensitive to acute DCA exposure compared to other species tested and reported in the literature. Reduced survival, as well as cardiac, developmental, and morphometric alterations were observed at concentrations as low as 8  $\mu\text{g/L}$ . Large interspecies variation in sensitivity to chemicals needs to be considered when performing environmental risk and impact assessment, and toxicity data on relevant species should be selected.

### **1.07.P-Th031 Ecotoxicity of Perovskites as Multi-component Advanced Materials (AdMs) in Aquatic Environments: from Cellular Effects to Acute Toxicity and Bioaccumulation Potential in Fish**

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Perovskites can exist with specific crystal structures and unique chemical compositions, leading to their tunability for applications in various industries. Intelligent testing strategies are required to meet regulatory demands for the safe use of such advanced, multi-component nanomaterials. Since fish represent model organisms for aquatic ecotoxicity testing and there are standardised test guidelines (TGs) based on these organisms, one of the objectives of this work was to assess the applicability of such TGs in a testing strategy for evaluating the toxicity of these advanced materials. With this in mind, the rainbow trout (*Oncorhynchus mykiss*) RTgill-W1 cell line acute toxicity test (OECD TG 249), the fish 96h acute toxicity test (OECD TG 203) and the fish bioaccumulation test (OECD TG 305) were used. The use of the RTgill- W1 cell line provided a high throughput approach to material testing. It acted as a predictive screen and a tool for deeper mechanistic adverse outcome pathway (AOP) focused investigations. Adaptations to the standardised OECD TG 203 were incorporated (e.g. use of turbines and humic acid) to facilitate material exposure concentration maintenance during testing with juvenile rainbow trout. While material stability was overcome by applying the dietary exposure route to test material bioaccumulation using TG 305.

Lanthanum-nickel-cobalt perovskites were tested, as well materials doped with Pt and the rare earth metal Pd, using the mentioned strategy. As well as providing much needed ecotoxicological information, collectively the information generated fed into a safe and sustainable by design (SSbD) approach ranking material hazard potential. Links were made between the materials physico-chemical properties and effects. Single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) was used to analyse fish tissues for different accumulation patterns. By measuring the uptake and depuration of specific perovskite metal components, differences in potentials for accumulation were identified. Also fish were analysed for sublethal biomarkers of toxicity associated with potential underlying mechanisms of chronic toxicity (e.g. oxidative stress). This developed strategic AOP directed integrative approach on testing and assessment (IATA) using fish as a model organism for aquatic toxicity testing will be used in the decision support tool being developed within the EU Horizon 2020 project HARMLESS grant agreement No. 953183.

### **1.07.P-Th032 Effect of Psychoactive Compounds on Neurotransmission in Fish**

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Psychoactive compounds, including psychiatric pharmaceuticals and illicit drugs, are emerging contaminants in the aquatic environment. They are introduced to the aquatic ecosystem either as parent compounds or their metabolites through improper disposal, human urine and feces. Furthermore, these compounds have been found in aquatic organisms, noticeably in fish. The neurotoxicity of psychoactive substances is revealed in fish behavioral alteration, hyperactivity and hypoactivity. Moreover, psychoactive substances cause reproductive toxicity, including altered sperm morphology and decreased courtship and reproductive output in fish. This study aims to observe the changes in neurotransmitters concentrations in fish as a response to exposure to psychoactive compounds. Bioaccumulation of psychoactive drugs, as well as histological changes and sperm motility, were also investigated. The experiment was conducted in the laboratory under controlled conditions in 200 L aquaria with adult males of European Perch (*Perca fluviatilis*). A mixture of seven psychoactive compounds (citalopram, donepezil, gabapentin, methamphetamine, sertraline, tramadol, venlafaxine) was used for the study at the final concentration of 1 µg/L and introduced to each aquarium, excluding control aquaria, which were maintained in drug-free water. Water samples (n = 180) were taken during the test in 24-hour intervals before and after water change in the aquaria. The samples were analyzed using the in-line-SPE-LC/MSMS method. The actual concentration corresponded to the nominal with the average difference of 18% for all compounds except of sertraline, for which a significant concentration decrease after 24 hours was observed. Fish were exposed to the target psychoactive compounds for 23 days, and sampling was conducted in 0-, 7- and 23-day. Specifically, fish brains, eyes, gonads and sperm samples were analyzed for the selected neurotransmitters and for psychoactive drugs using LC/HRMS/MS. Information about contaminant-induced changes in neurotransmitters can be crucial because it may link behavior and physiology in the exposed fish.

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### **1.07.P-Th033 Effects of Tributyltin Exposures in Zebrafish Embryos by Multi-omics**

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The Zebrafish (*Danio rerio*) has emerged as a powerful model organism across a broad range of scientific fields, including ecotoxicology. The Zebrafish model presents various advantages over other common model organisms such as its effortless manipulation or large offspring. Strikingly, toxicological data from zebrafish can be extrapolated not only to aquatic species but also to other vertebrates, including humans. Furthermore, zebrafish embryos are considered an excellent alternative animal

model with fewer ethical restrictions, ensuring compliance with 3R's principle (Replacement, Reduction, and Refinement) in animal research. As a result, their use in ecotoxicological studies particularly in endocrine-disrupting chemical (EDCs) assessment has gained recent importance.

While single-omic technologies have been widely used in ecotoxicology to identify molecular disruptions induced by environmental pollutants in target samples, they are specific to a single biological layer, leading to overly simplistic approaches that provide a limited understanding of complex molecular pathways and biological processes. In recent years, multi-omic approaches have gained significant attention due to their potential to provide a more comprehensive understanding of underlying mechanisms and pathways in biological systems. Therefore, the integration of multi-omic analysis is a powerful tool to efficiently capture not only the toxic mechanisms but also multifaceted networks from gene to phenotype.

In the present work, we investigated the endocrine-disrupting behavior of Tributyltin (TBT), an organotin compound known to cause significant environmental consequences. To accomplish this, we conducted a multi-omic analysis combining untargeted LC-HRMS lipidomics and RNA-Seq transcriptomics data after exposing zebrafish eleutheroembryos to environmentally relevant concentrations (from 0.3 to 100 nM) from four to five days post fertilization. The results obtained from the multi-omic pathway enrichment were in concordance with the main single omics results. Interestingly, the multi-omic analysis also revealed the disruption induced in pathways that were not found significantly affected in the single-omic studies. To confirm the accuracy of the multi-omic, we conducted complementary behavioral testing and neurotransmitter analysis. Collectively, this study demonstrates the usefulness of multi-omic data analysis in better understanding the modes of action of EDCs.

#### **1.07.P-Th034 Effects of a Reduced Graphene Oxide-Silver Nanoparticle Hybrid Material on Zebrafish Embryos**

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The hybrid material consisting of reduced graphene oxide and silver nanoparticles (rGO-Ag) is one of the most promising compounds for optoelectronic, catalytic and electrochemical applications. Taking into account the nanosize of this material and its large surface area, possible ecotoxicological and environmental safety aspects are raised. The aim of this study was to analyze the hazard of rGO-Ag in zebrafish embryos and to compare them with the effects caused by other silver and graphene family compounds. Newly fertilized embryos were exposed for 120 h up to 2.5 mg/L of rGO(PVP)-Ag and to the equivalent concentrations of ionic silver and Ag nanoparticles (size range: 5-50 nm; mean size: 17 nm), as well as to the rGO-Ag filtrate in order to study the effect of potentially dissolved silver. Developmental parameters (survival, hatching and malformations) were monitored along exposure and effects on cell death and on catalase and acetylcholinesterase (AChE) activities were measured at the end of the exposure. Acute toxicity tests showed that the LC50 value for the rGO-Ag (0.306 mg/L) was 12 and 7,5 times higher than the LC50 value for ionic silver (0.025 mg/L) and for Ag nanoparticles (0.041 mg/L), respectively. The LC50 value of filtrated rGO-Ag could not be calculated as it was higher than tested concentrations, indicating a low contribution of dissolved silver ions to the observed toxicity. Moreover, at equivalent concentrations, higher fluorescence intensity revealing cell death was observed in embryos exposed to ionic silver and Ag nanoparticles than in those exposed to rGO-Ag. Increased malformation prevalence was only recorded at the highest concentration of rGO-Ag filtrate, but at concentrations  $\geq 0.156$  mg/L of rGO-Ag. Both silver forms provoked malformations, especially yolk sac edema, at lower concentrations. No significant changes were recorded in catalase activity. Exposure to 3.8 and 7.7  $\mu\text{g/L}$  of Ag nanoparticles, but not of ionic silver, and to 0.156 mg/L of rGO-Ag significantly inhibited AChE activity. These results demonstrated that the hybrid compound rGO-Ag is less toxic than ionic silver and than Ag nanoparticles, but still can pose a risk for aquatic organisms. \*Work funded by Basque Government (consolidated research group IT1302-19 and IT1743-22) and Spanish MINECO project NACE (CTM2016-81130-R).

#### **1.07.P-Th035 Effects of exposure to Piriproxifen on the survival of adult *Danio rerio* (Zebrafish)**

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Considering the great concern about controlling the proliferation of vectors that reproduce in aquatic environments, the adoption of larvicides in water reservoirs for various purposes has become increasingly frequent. Among the active ingredients recommended by the WHO for use in drinking water is Pyriproxyfen (PPF). The most common risk assessment procedures use occurrence and toxicity data on pesticide active ingredients. Therefore, it is necessary to evaluate the real harmful effects of these substances that are increasingly present in aquatic environments. The model based on *Danio rerio* - zebrafish has become popular for toxicity assessment, mainly because it is an organism with anatomy, physiology and development similar to higher vertebrates and because it is representative and recognized as a model for aquatic environments. Thus, our objective was to identify the possible toxic effects resulting from acute exposure of *Danio rerio* adults to PPF, aiming to obtain the LC50 for this molecule. For this study, fish of the AB lineage were used, from the fish facility of the Federal University of São Paulo, with 3 months of age, and for each exposure, replicates were carried out with 10 individuals each. PPF was solubilized with the help of DMSO. The first concentrations tested were: 0.0001, 0.001, 0.01, 1, 5 and 10 mg/L. Only concentrations of 5mg/L and 10 mg/L induced mortality of 60% and 86.6%, respectively. In a second experiment, concentrations of 2, 3 and 4 mg/L were selected, and all of these concentrations presented similar mortality percentages (63.3%, 60% and 56.7%, respectively). In a

third stage of experimentation, concentrations of 2, 4 and 6 mg/L were tested, which were capable of inducing mortality rates of 62.5%, 60% and 87.5%, respectively. As a final step, we carried out the tests using concentrations of 1; 1.5; two; 2.5; 3; 3.5 and 4 mg/L. In this final experiment we obtained mortality percentages from 0 to 1 mg/L; 10% to 1.5; 60% for 2 and 2.5 mg/L; 62.5% for 3 mg/L; 60% for 3.5 and 4 mg/L. Our results do not allow us to obtain a reliable LC50 value, however it is possible to infer that the LC50 of PPF is between concentrations of 1.5 and 2 mg/L. The impossibility of precisely estimating the LC50 of PPF is probably due to solubility problems of the compound.

#### **1.07.P-Th036 Effects of the thyroid hormone system disrupting compound metformin on the development of early life stages of zebrafish (*Danio rerio*)**

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Metformin is the most widespread antidiabetic drug treatment in the world, and with the increasing number of diabetic patients, an increasing production and consumption of metformin follows. Due to the incomplete uptake and metabolism of metformin when ingested, as well as insufficient wastewater treatment, metformin is released into the environment. In Germany, metformin has been detected in surface waters at an average concentration of 467 ng/L. Worldwide, metformin is amongst the top three most frequently detected pharmaceuticals in aquatic environments and has been shown to cause endocrine disruption in fish. Although, knowledge about the long-term effects of metformin on aquatic organisms is limited. To investigate effects of metformin on aquatic organisms, zebrafish (*Danio rerio*) will be used as a model organism, focusing on thyroid hormone system (THS) sensitive endpoints, such as swim bladder inflation and eye histopathology. It is known that THS is involved in numerous developmental processes, and that exposure to thyroid hormone system disrupting chemicals (THSDC), can have detrimental long-term effects on several organisms. Assessment of the effects of metformin on THS will be done with experiments based on the OECD TG 236: Fish embryo Acute Toxicity Test, testing concentrations of metformin that are environmentally relevant and focusing on sublethal and behavioral endpoints. Results from this study will contribute to a better understanding of the potential thyroid disrupting mechanisms of metformin on aquatic organisms, and how this might affect aquatic ecosystems.

#### **1.07.P-Th037 Effects of three alkyl-organophosphate flame retardants on neurobehavioral development and oxidative stress in zebrafish larvae**

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While concerns are growing on the presence of alkyl-organophosphate flame retardants (alkyl-OPFRs) in the environment, there is significant lack of knowledge on their potential toxicity. In the present study, we evaluated the neurobehavioral development and oxidative stress induction of trimethyl phosphate (TMP), triethyl phosphate (TEP), and tris(2-butoxyethyl) phosphate (TBEP) using zebrafish embryo/larvae. Zebrafish embryos were exposed to TMP, TEP, and TBEP (0, 0.02, 0.2, 2, 20, and 200 mg/L) for 96 h, and changes in developmental parameters, locomotor behavior, reactive oxygen species (ROS), and antioxidant enzymes were measured. TBEP exposure reduced body length and weight of larvae, whereas TMP and TEP did not significantly affect developmental parameters. Hypoactivity, decreased levels of acetylcholinesterase and dopamine, and down-regulation of genes involved in central nervous system (e.g., *ache*, *gap43* and *mbpa*) were observed in fish exposed to TEP and TBEP. The levels of ROS, superoxide dismutase, catalase, and glutathione peroxidase were significantly increased in fish exposed to TEP and TBEP. Pretreatment with N-acetylcysteine, the classic antioxidant, alleviated TEP and TBEP-induced neurobehavior toxicity and oxidative stress. The results of this study revealed that zebrafish locomotor behavior impairments and developmental delay induced by TEP and TBEP exposure are attributed to oxidative stress.

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Keywords: Alkyl-organophosphate flame retardants, Neurotoxicity, Oxidative stress, Zebrafish

#### **1.07.P-Th038 Embryotoxicity assessment of Diflubenzuron commercial formulations using the zebrafish model system**

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Diflubenzuron (DFB), a synthetic benzoylurea larvicide, is a chitin inhibitor used to control arthropods, including insect vectors of human and veterinary diseases. It is currently used to control insect infestations in agriculture and livestock and for ectoparasite control. Although this active ingredient is expected to have low toxicity to non-target species, unknown components in its commercial formulations, such as excipients, adjuvants, and surfactants, may contribute to or interfere with its toxicity. To understand the environmental impact of DFB applications, the most relevant approach is to test the commercial formulations due to the lack of information on their ingredients. In this study, we evaluated the toxicity of two formulations of DFB: (i) an oral formulation for bovine use and (ii) a water-soluble formulation for agricultural use, the latter tested by direct water dilution or using DMSO as co-solvent. Embryotoxicity was evaluated according to OECD Test Guideline No. 236. Zebrafish embryos up to 4 hpf were exposed to different concentrations of the formulations and the active ingredient (0.025, 0.125, 0.25, 1.25, 2.5, and 10 mg L<sup>-1</sup>) for 120 h (extended FET) to assess biomarkers related to development, morphological

changes and cardiotoxicity, and for 168 h to assess their effects on larval respiration rate (prolonged exposure until larvae are large enough for measurement). For the statistical analysis, the assumptions of normality and homoscedasticity were tested. Parametric data was compared using one way ANOVA/ Tukey and non-parametric data was compared using Kruskal-Wallis/DSCF. Our results showed that both formulations caused an increase in larval heart rate ( $p < 0.001$ ) and changes in respiratory rate ( $p < 0.05$ ), indicating the possible influence of these formulations on larval metabolism. In addition, larvae embryos to the water-dispersible formulation (containing DMSO) at  $10 \text{ mg L}^{-1}$  showed an increase in notochord malformations compared to the control group ( $p = 0.042$ ). To date, we have performed an exploratory assessment of the potential teratogenic effects induced by commercial formulations of DFB on zebrafish embryos, and the effects were associated with sublethal changes observed under acute exposure. Thus, our results provided insights into the potential environmental impact of DFB formulations. In the next steps of this work, we will investigate effects at the cellular level, as well as tests on adult individuals.

#### **1.07.P-Th039 Exploiting Zebrafish Tools for a Safe and Sustainable Development of Nano-Enabled Antimicrobials to Reduce the Presence of Antimicrobial-Resistant Bacteria in the Aquatic Environments**

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Nanoparticles (NPs) and nano-enabled products (NEPs) emerged as novel antimicrobial agents with proven efficacy against antimicrobial-resistant bacteria polluting aquacultures. Nevertheless, their safety and sustainability must be evaluated at an early design phase.

This work aims at evaluating the safety and sustainability of a platform of nano-antimicrobials in exploitation scenarios by using zebrafish (*D. rerio*) as model organism for high-throughput developmental and behavioural screening, in parallel with the Life Cycle Assessment (LCA) methodology.

The study focused on CuO NPs-nano-engineered antimicrobial/biofilm fish cage nets and water filtration membranes, as well as on marine-derived nano-formulated antimicrobial agents, proposed as an alternative to antibiotics for fish. Specifically, the study investigated water-based copper oxide (wCuO) NPs and wCuO-based NEPs, including coated, co-extruded polymers and coated filtration membranes, along with nano-formulations of microalgae-derived lipid NPs and quorum quenching enzymes. A definition of new protocols for the leaching of nanomaterials was attempted and is here proposed. The NPs suspensions and leachates from the NEPs were characterized by TEM, DLS and ICP-OES, while their aquatic toxicity potential was assessed using the Fish Embryo acute Toxicity test (OECD n.236). The Lethal Concentration 50 (LC50) and the Effective Concentration 50 (EC50) were calculated. Morphometric and behavioural analyses were performed on exposed embryos to assess additional indicators of sub-lethality.

LCA studies were carried out to assess the environmental sustainability of the materials' production. The associated impacts were assessed with the CML 2001 impact method and the OpenLCA software. Within the inventory phase, primary data and information on the synthesis/production processes of the NPs and the NEPs were collected and modelled.

Toxicology analyses did not highlight any significant lethal effect for all the NEPs and the hatching was the most affected endpoint at sub-lethal level. The LCA analyses identified the most impactful steps within the materials' production.

The integration of nano-ecotoxicology assessment and standard LCA studies is suggested as an effective methodology to provide a comprehensive and harmonised framework for decision-making during the design stage of safe and sustainable nano-enabled antimicrobials, for example, within the field of fish farming.

#### **1.07.P-Th040 Filling The Fish Toxicity Data Gap of Pesticides Using in silico Tools:**

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Pesticides are a common group of chemicals used to facilitate human life in many areas. In 2018, the total amount of pesticide use in Turkey increased by 10.9% compared to 2017 and had reached 60,020 tons. Aquatic toxicity is one of the major concerns since the life cycle of chemicals mostly ends up in the water compartment. Considering all types of pesticides and the damage they cause to the environment, the need for toxicological and ecotoxicological data is very high. It is almost impossible to allocate enough time, budget and chemical use for the experimental testing required to fill this data gap. For this reason, substitution methods are used for the replacement of laboratory tests (i.e., *in vitro*, and *in vivo* methods) with alternative methods (i.e., non-experimental *in silico* methods) like quantitative structure-toxicity relationships (QSTRs). QSTR models for *Oncorhynchus mykiss* toxicity predictions of pesticides are notably depends on a small dataset, without using 3D descriptors or do not fulfill the OECD validation requirements. In the presented study, robust and reliable quantitative structure-toxicity relationships (QSTRs) for the toxicity of pesticides to fish with a defined applicability domain were generated and fish toxicity of pesticides with no experimental data was predicted by using the novel QSTR models.

For model development, experimental aquatic freshwater toxicity data of pesticides (from reliable sources which showed compliance with OECD principles for testing of chemicals) for fish were gathered from several databases and literature. Both for external validation and to fill the gap in the literature, the missing data for fish were estimated using these models. The chemicals within the AD of each model were listed. Validated QSTR models based on the Ordinary Least Square method were developed for acute toxicity prediction towards *Oncorhynchus mykiss*. Robust and predictive QSTR models were using 313 pesticides' toxicity data. The importance of 3D descriptors for pesticides in QSTR models for fish was underlined. The presented QSTR models were used to fill data gap for more than 300 pesticides without fish toxicity, by providing wider

applicability domain than the existing studies in the field. The generated fish toxicity QSTR models for pesticides has a potential to be used in screening and prioritization of pesticides.

#### **1.07.P-Th041 Harmful Effects of Caffeine on the Fish *Poeciliopsis gracilis* Heckel, 1848 (Poeciliidae)**

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*Poeciliopsis gracilis* is a species native to Mexico, it lives in lentic areas of rivers and streams in the tropical portion of the coasts of the Gulf of Mexico and the Mexican Pacific. Because there are no studies on the response of *P. gracilis* to pollutants, in this study an evaluation of the effects on survival and physiological rates (respiration rate, excretion rate, feeding rate and growth rate) was carried out: in juvenile fishes exposed to caffeine. Initially, an acute bioassay was carried out where six concentrations of caffeine (0.0, 0.5, 1.0, 5.0, 10.0, 15.0, 20.5 mg/L) were tested (n = 10 in triplicate), to determine the lethal concentration (LC<sub>50</sub>) (Probit) to 96 hours. Subsequently, a test lasting 20 days was carried out. Fish were exposed to 2 sublethal concentrations (LC<sub>50</sub>/100 and LC<sub>50</sub>/10). Samples were taken at 5, 15 and 20 days to measure physiological rates and at the end of the exposure period the growth rate was evaluated. The LC<sub>50</sub> obtained in the acute bioassays was 14.1 mg/L. In the sublethal bioassays, an increase in the respiration rate was observed in the first days of exposure and subsequently a decrease. On the contrary, the excretion rate increased throughout the exposure time. The feeding rate decreased after 15 days of exposure, consequently the rate of weight gain was lower compared to the control group. The O:N ratio obtained showed that the organisms presented high stress after 15 days of exposure to caffeine. The data obtained demonstrate that caffeine causes harmful effects to juveniles of *P. gracilis*.

#### **1.07.P-Th042 INTOB: Advancing Digital Infrastructure for Enhanced and findable, accessible, interoperable, and reproducible (FAIR) Management and Analysis of Toxicological Data Obtained with Zebrafish Embryo**

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Toxicological testing plays a pivotal role in diverse contexts, ranging from regulatory approvals for chemical substances to environmental assessments and scientific investigations into toxicity's cause-and-effect relationships. Here we introduce INTOB (Integrated effect data base for toxicological observations on organism scale), a software designed for the structured management of toxicological data and metadata according to the FAIR principles ("findable, accessible, interoperable, and reproducible"). INTOB facilitates the FAIR management of observation data generated through the widely employed zebrafish embryo toxicity test, a recognized OECD standard (OECD TG 236). The application extends to areas such as the quality assessment of wastewater in accordance with DIN EN ISO 15088.

INTOB serves as a comprehensive solution, its central in-house database not only ensures data sovereignty for users, but also provides a robust system for quality control and standardisation, and flexibility in data usage. In a landscape where the digitization of toxicological observations is still in its infancy, INTOB emerges as a state-of-the-art solution, addressing the urgent need for efficient data management in response to political, regulatory, and industrial demands. Furthermore, INTOB sets the stage for the integration of artificial intelligence-based analytical methods in the realm of toxicology, presenting a forward-looking perspective on the evolution of toxicological data management and analysis. Here we explore the functionalities, benefits, and potential applications of INTOB, offering valuable insights into the future trajectory of digitization in toxicology.

#### **1.07.P-Th043 Identification of Differences in Mechanisms of Developmental Neurotoxicity of Methyl-, Ethyl-, and Propyl-Parabens in Zebrafish Embryos Through Transcriptome Analysis**

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Para-hydroxybenzoates, including methyl, ethyl, and propyl parabens, are widely used industrial preservatives known for their antiseptic properties. However, their chemical nature allows easy release into indoor environments during product use, posing concerns for infants and young children who spend extended periods indoors. This study employed a zebrafish embryo model to assess the neurotoxicity of commonly encountered methyl, ethyl, and propyl parabens.

In response to the limited comparative developmental (neuro)toxicity studies on parabens, the investigation aimed to align phenotypic observations with transcriptomic analyses. Zebrafish embryos (2–4 hours post-fertilization) were exposed to varying concentrations (0, 5, 10, 20, 40, 80, 150, and 300 µM) of the parabens. Acute toxic effects were assessed through embryonic mortality-based POD (M-POD) values at 120 hpf, utilizing a 5% benchmark dose. Behavioral assessments were conducted on 120 hpf zebrafish at M-POD concentrations of 0, M-POD/50, M-POD/10, and M-POD, with behavior analyzed using Zebrabox. Transcriptome analysis at 120 hpf after exposure to sublethal paraben concentrations (0, M-POD/50, M-POD/10, and M-POD) provided tentative transcriptome-based POD (T-POD) values for each paraben, compared with M-POD values.

In conclusion, long-chain propyl paraben exhibited greater acute toxicity than short-chain methyl and ethyl parabens. Behavioral assays at BMD 5 concentrations, determined based on morphological incidence, revealed changes in larval behavior only in the ethyl paraben-exposed group. Subsequent transcriptome analysis confirmed that the observed

hyperactivity was associated with derangement. T-POD values for the three parabens were similar, despite differences in embryotoxicity and malformation based on chain length. This suggests that adverse effects of the three parabens can manifest at similarly low concentrations. However, distinct molecular perturbations determine varying degrees and consequences of toxicity. The study demonstrates that POD-based transcriptome alterations are effective for comparing molecular mechanisms regulating phenotypic traits between two or more toxic chemicals.

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#### **1.07.P-Th044 Identification of Molecular Markers on Zebrafish Embryo for Thyroid Disruption by Transcriptomic Analysis**

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Environmental pollution is a rising concern for both human and environmental health. Among others, the evaluation of endocrine disruptor chemicals is a challenge for both regulators and industry. Only a few methods exist to identify Thyroid Disruptor Chemicals (TDC). Those methods are low throughput and expensive or do not often comply with the animal testing ban of cosmetic regulation. Therefore, it is becoming urgent to develop novel strategies and screening methods to identify TDCs. As thyroid signaling is highly conserved between teleost and mammals, zebrafish embryo is an alternative model for studying both physiological regulations and disruption. We design a transcriptomic analysis where embryos are exposed to reference compounds alone or in combination with thyroid hormones (T<sub>3</sub> and T<sub>4</sub>) according to the FET test protocol (Fish Embryo Test - OECD guideline, n°236). We selected four reference compounds: Iopanoic acid (IOP), Sodium Perchlorate (PCL), Tetrabromobisphenol A (TBBPA) and Propylthiouracil (PTU) based on their different modes of action on the thyroid signaling pathway. We choose to expose the embryos at dose corresponding to FET EC10 and analyzed their transcriptomes by RNA sequencing.

After quality control, reads were mapped to *Danio rerio* genome. Lists of differentially regulated genes (DEGs) are classified in several clusters, each corresponding to a type of biological response. Despite a great diversity of biological responses, DEGs are classified into three families: chemical dependent (Only compound), Thyroid Hormone dependent (TH only) and crosstalk responses (Potential TD). These correspond to specific effects due to the action of selected compounds alone or in co-treatment with thyroid hormones (crosstalk responses).

We found major effects with our reference compound alone, but virtually no effect with THs alone. Co treatment TH + compound revealed not independent responses thereby identifying potential thyroid signaling disruption. The biological processes affected are related to apoptosis, metabolism, and immune system. We provide a list of around 80 potential biomarkers of thyroid signaling disruption.

#### **1.07.P-Th045 Impact of Trifloxystrobin Exposure on the Early-Stage Zebrafish (*Danio rerio*) Metabolome**

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Trifloxystrobin (TFS) belongs to the strobilurin group of fungicides widely used in agriculture. TFS is known for its broad-spectrum activity against a wide range of fungal pathogens. This fungicide inhibits the mitochondrial respiratory chain complex III in fungi, disrupting their ability to generate energy. TFS was found to cause severe developmental toxicity in zebrafish (*Danio rerio*) embryos, resulting in developmental delays as well as heart and vascular defects. As the alterations in metabolic pathways induced by TFS are still unknown, we carried out metabolomics to identify developmental toxicity. After being treated with TFS 0.2 mg/L to zebrafish embryos up to 96 h post-fertilization (hpf). Phenotypes were photographed at the endpoint and samples were collected for analysis of metabolites. Embryos exposed to TFS showed various phenotypes like curved spine, yolk sac edema, and heart edema at 96 hpf. Relative amounts of compounds that were included in metabolic pathways were confirmed with GC-TOF-MS. Based on the results of multivariate statistical analysis, the control group and TFS-treated group were well distinguished. 42 metabolites were selected and identified by the OPLS-DA model, and the amounts of glutaric acid were relatively increased in the TFS-treated group. Compared to the control group, the TFS-treated group showed relatively lower fatty acid and its derivatives. Taken together, TFS exposure can lead to the disruption of various pathways in developing zebrafish embryos with potential implications for carbohydrates and fatty acids in organisms. These findings indicate that the application of TFS in the agricultural field could potentially have adverse effects on the early-stage development of organisms in aquatic ecosystems.

#### **1.07.P-Th046 Investigating The Mechanisms of Endocrine Disrupting Chemical-Induced Masculinization in Medaka (*Oryzias latipes*)**

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Endocrine disrupting chemical (EDC)-induced sex disruption in fish is blamed to severely reduce fish populations and cause critical effects to aquatic ecosystem [1]. However, the molecular mechanisms of chemical induced sex disruption in fish are



not well-understood yet. Recent studies indicate that late-embryonic to early larval stages are the critical period for gonad differentiation in medaka (*Oryzias latipes*). The sex of medaka is basically determined by genotypes (XX/XY) and the critical sex determination gene, *DMY/dmrt1bY*, is located on Y chromosome [2]. Meanwhile, some key genes expressed in the critical period for gonad differentiation are also identified in medaka. The aim of this research is to investigate the mechanisms of chemical induced sex disruption in gonad differentiation during late-embryonic to early larval stages of medaka after exposure to synthetic sex hormones. Candidate genes at early stages are firstly selected by comparing the expression levels in XX and XY medaka larvae. Next, known EDCs, such as 17 $\alpha$ -methyltestosterone (MT), are applied for investigation of the molecular mechanisms of chemical induced sex disruption in medaka. In addition, we will further discover novel genes by RNA-seq to depict the molecular mechanisms of chemical induced sex disruption in medaka.

#### **1.07.P-Th047 Is the Japanese Medaka a Reliable Species for Testing Endocrine Disrupting Compounds?**

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The Japanese medaka *Oryzias latipes* is one of the recommended species in various internationally validated guidelines for the testing of chemicals to assess fish toxicity, including those developed for identifying the potential of substances for endocrine disruption (ED). Several of these tests are exclusively conducted with this species (OECD TG 240: Medaka extended one generation reproduction test - MEOGRT; OECD TG 251: Rapid Androgen Disruption Activity Reporter Assay – RADAR; OECD Series on Testing and Assessment GD 379: Juvenile medaka anti-androgen screening assay - JMASA). In the following, the pros and cons for using the Medaka in ED testing are discussed.

There are various reasons for the popularity of medaka. First of all, it is an easy-to-breed species in laboratory conditions, and it has been used for so long in testing that all its requirements are well-known and well handled. Moreover, it is small enough to allow testing on a sufficient number of fish for statistical robustness while being large enough to allow a detailed analysis of several parameters. The duration of its life cycle with an early sexual maturity at 2-3 months, is compatible with laboratory experimentation, and results can be expected in a reasonable time. Finally, the species is characterized by easily identifiable secondary sex characteristics in the male individuals, explaining the interest in this species for ED testing.

Based on such elements, this species could be the perfect choice for conducting testing for the identification of ED compounds, and that is probably why it is recommended in so many guidelines. Then why could the use of medaka in ED testing be questioned? The fact is that under some breeding conditions, medaka may undergo sex reversal from female to male without any exposure to any xenobiotic compound, during early development. The effect of temperature on the potential for sex reversal has been indeed known for more than a decade and is briefly mentioned in the JMASA guideline introduction. But more recent papers indicate that other stress factors could also induce sex reversal.

Therefore, since sex reversal can be induced without direct interaction of the tested chemical with an EAS modality, positive results in tests involving developmental phases such as MEOGRT and JMASA, but also FELS test (OECD 201: Fish early-life stage toxicity test), and fish full life-cycle test (EPA OPPTS 850.1500) should be considered with care.

#### **1.07.P-Th048 Metabolomics approach to detecting toxicity in *Oreochromis niloticus* exposed to natural and synthetic organophosphates**

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The proliferation of toxic cyanobacteria, caused by the intensification of the eutrophication process, generally coincides with pesticide pollution, mainly by surface runoff and leaching. Therefore, this study aimed to evaluate the toxicity of the cyanobacterium *Sphaerospermopsis torques-reginae* (ITEP-024 strain), which produces a natural organophosphate (guanitoxin) associated with synthetic organophosphate pesticides in specimens of *Oreochromis niloticus*. For this, we verified the exposure of *O. niloticus* for 96 h to six treatments, being: control group (C), aqueous extract of ITEP-024 250 mg/L (T1), insecticide malathion 1 mg/L (T2), insecticide trichlorfon 0.5 mg/L (T3), ITEP-024 + malathion (T4), ITEP-024 + trichlorfon (T5), and ITEP-024 + malathion + trichlorfon (T6). To detect the deleterious effects on fish, we used a global metabolomics approach with the quantification of primary metabolites by UHPLC-MS/MS. Besides that, an Integrated Biomarker Response - version 2 (IBRv2) was employed to correlate the response of the metabolites in the treatments to the control group. Thirty-nine primary metabolites were quantified, including amino acids, nitrogenous bases of DNA, nucleosides, and organic osmolytes. Among them, 11 showed a down or up-regulation. Proline, glycine, alanine, histidine, serine, aspartate, and guanosine presented a downregulation, while carnitine, cystine, thymine, and trimethylamine N-oxide (TMAO) showed an up-regulation. The higher IBRv2 values were in the T4 and T2, indicating the most deregulation for both treatments. Amino acid deregulation may impair vital organism activities, even as changes in thymine levels can affect DNA integrity and gene expression, which can have several negative consequences for organisms. Furthermore, guanosine has several biological functions, and the decrease in the content suggests inhibiting the organism's cellular growth activity. In conclusion, this study represents the first attempt to assess the synergistic interactions between guanitoxin-producing cyanobacteria and insecticides. We indicated that the combination of malathion and guanitoxin-containing extracts was particularly concerning. Despite the importance of observing these metabolites in ecotoxicological assessments, there is a need for studies examining their concentrations in fish

following chemical exposure, indicating the urgency of understanding how these metabolites can be deregulated and how aquatic animals cope with this damage.

#### **1.07.P-Th049 Nanoplastic-induced toxicity mechanism in intact zebrafish larvae revealed by novel magnetic resonance method**

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Considering the ubiquitous presence and enduring nature of plastics globally, exposure to these materials, especially nanoplastics (NPs), is unavoidable for both humans and animals. Polyethylene terephthalate (PET) is one of the most widely used plastics. Despite the widespread use of PET in packaging, currently, limited research exists on the bioaccumulation and toxicity of nanoparticle originating from PET (PET NPs). Moreover, due to lack of appropriate methods, the system-level understanding of toxicity mechanism of PET NPs is not known. The present study aimed to systematically investigate the physiological and metabolic effects of PET NPs exposure and to explore the underlying toxicity mechanism. To elucidate systematic understanding of the toxicity mechanism of PET NPs we utilized state-of-the-art high-resolution magic angle spinning nuclear magnetic resonance (HRMAS NMR), applied to intact zebrafish (*Danio rerio*) embryo. Accumulation of PET NPs in larvae was observed in the liver, brain as well as intestine which coincides with the localization of reactive oxygen species in this area. HRMAS NMR based metabolic profile of zebrafish coupled with multivariate analysis revealed liver impairment caused by PET NPs. Targeting of liver by PET NPs was also indicated by an increase in various potential biomarker of the hepatotoxicity. HRMAS NMR spectra also clearly show elevated levels of fatty acids as well as polar head groups of phospholipids, signifying PET NPs induce disruption of membrane integrity. Furthermore, metabolites associated with cellular bioenergetics (e.g., glucose, lactate, acetate, ATP and NADH) were significantly affected by PET NPs exposure. Taken together, our work provides a comprehensive system level insight into the mechanism of PET NPs induced toxicity.

#### **1.07.P-Th050 Neurodevelopmental Effects of Petroleum Exposure on Developing Zebrafish (*Danio rerio*): Insights into Sensory-, Motor- and Anxiety- Responses**

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Oil spills cause serious threats to aquatic ecosystems and are very challenging to assess due to the highly complex and unique composition of oils. Studies demonstrated that developing fish exposed to crude oils were particularly susceptible to adverse effects at low concentrations, which can have severe consequences for entire populations. In this context, our previous work showed a structural impairment of the visual system and first indication of developmental neurotoxicity (reduced spontaneous tail coiling) in oil-exposed zebrafish. However, the impact on other sensory organs such as the lateral line system (LLS) and a detailed understanding of different neurotoxic pathways remains largely unknown. Hence, the present study aimed to investigate whether water-accommodated fractions (WAF) of native and chemically dispersed crude oil and chemical dispersant alone impact 1) the development of the LLS, 2) the motor neuron development, and 3) anxiety-like behavior in zebrafish embryos. Embryos were exposed to sublethal effect concentrations of WAFs ( $\leq EC_5$ ) below the threshold for visible morphological malformations. For posterior LLS assessment, potential apoptotic hair cells and hair cell damage were quantified using *in vivo* double-staining with DAPI (nuclei) and DASPEI (mitochondria) at 96 hours post fertilization (hpf). Using confocal laser scanning microscopy, examination of *in vivo* embryonic motor development was performed by focusing on primary motor neurons in a double-transgenic zebrafish line (24-48 hpf), highlighting caudal primary motor neurons expressing the GFP-bound receptor neuropilin 1a (*nrl1a:eGFP*), in a pan-neuronal reporter background (*xla.tubb:DsRed*). As an indicator for anxiety-behavior we studied thigmotaxis in 120 hpf zebrafish assessed by stimulating thigmotactic (wall-hugging) behavior using an external stressor (sudden darkness). Preliminary results indicated increased thigmotaxis after petroleum exposure and a trend towards increased relative axonal motor neuron length at 24 hpf after chemically dispersed crude oil exposure. We expected a higher responsiveness of the hair cells after WAF exposure due to the direct interface with the surrounding medium. Overall, the present results will help to provide a more holistic profile of neurodevelopmental toxic effects and potential modes of action after petroleum exposure.

#### **1.07.P-Th051 Neurotoxic effects of antidepressants on zebrafish embryos.**

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Increasing use of Benzodiazepines has been linked to an increase in anxiety and depression cases in the post-pandemic period. As they are able to avoid the most common purification treatments, it is reasonable to expect an increase of their concentrations in surface waters. The aim of the study has been to evaluate the ecotoxicological effects of benzodiazepine on zebrafish model, in particular the research focused on the neurotoxic effects on zebrafish embryos.

The active substance that has been chosen is alprazolam, a widely used benzodiazepine even among the younger population. Ecotoxicological data is currently unavailable and the Predicted No Effect Concentrations (PNEC) for fish was estimated by ECOSAR to be 18 µg/L.

Therefore, in order to obtain more informations, two ecotoxicological tests were applied and compared: the "Fish Embryo toxicity (FET) test" (OECD 236, 2013) and the neurotoxicity test "Coiling Activity of Tail (CAT) test". Thus, the tests were conducted at three concentration levels: 25 µg/L, 250 µg/L, and 2500 µg/L. The comparison of the results obtained with the ecotoxicological test showed that there were no significant acute effects with the FET since the maximum measured value (25%) was detected for concentrations of 250 µg/L and 2500 µg/L. The increasing trend of concentrations is described by the effects of sublethality. In particular, the CAT test showed a direct relationship with the concentrations analyzed. In addition, the effect percentage of the endpoint number of tail beats per minute (one of the three parameters measured with CAT), showed significantly higher values than the control already at the lowest concentration of alprazolam 25 µg/L.

In conclusion, the present study has shown the neurotoxic effect of alprazolam on zebrafish embryos, in particular the drug has determined hyperactivity for all the tested concentrations. Finally, it should be noted that the lowest concentration 25 µg/L is similar to the PNEC value 18 µg/L extrapolated and has determined important effects of neurotoxicity on embryos.

Therefore, more attention should be paid to the monitoring and effect of benzodiazepines in surface waters. Early warning systems such as CAT are also key and useful tools for detecting the effects of climate change. The upcoming harmonisation and standardization of this method would be necessary due to its potential value in implementing EU guidelines and regulatory strategies to identify neurotoxic risks in ecosystem.

**1.07.P-Th052 Osmotic diuretic exposure blocks aryl phosphate ester-induced pericardial edema in zebrafish embryos**  
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Pericardial edema (PE) – fluid accumulation within the pericardium – is a frequently observed malformation in zebrafish embryo-based chemical toxicity screens. We recently found that triphenyl phosphate (TPHP)-induced PE was mitigated by an increase in the osmolarity of exposure media, suggesting that PE may be driven by a disruption in osmoregulation across the embryonic epidermis. Therefore, the objective of this study was to investigate whether, similar to TPHP, PE induced by other structurally related aryl phosphate esters (APEs) is dependent on the osmolarity of exposure media. To accomplish this objective, we first mined the peer-reviewed literature to identify other APEs that 1) induced PE in zebrafish embryos within a minimum of 3 peer-reviewed publications and 2) demonstrated a statistically significant induction of PE in ≥60% of the studies. Based on this meta-analysis, we identified 5 other APEs that caused PE in zebrafish embryos: triisopropylated phenyl phosphate (IPP-3), cresyl diphenyl phosphate (CDP), tricresyl phosphate (TMPP), 2-ethylhexyl diphenyl phosphate (EDHP), and tert-butylphenyl diphenyl phosphate (BPDP). Using TPHP as a positive control and PE as a readout, we developed concentration-response curves for all 5 APEs based on a static exposure from 24-72 h post-fertilization (hpf). Using the EC50 concentration (based on PE) for each APE, we then conducted co-exposures with D-Mannitol (an osmotic diuretic) from 24-72 hpf to determine whether, similar to TPHP, an increase in the osmolarity of exposure media mitigated APE-induced PE at 72 hpf. Using PE as an endpoint, the approximate EC50s for TPHP, IPP-3, CDP, TMPP, and EDHP were 6.25, 3.125, 3.125, 25, and 100 µM, respectively. Interestingly, similar to our findings with TPHP, co-exposure with D-Mannitol from 24-72 hpf completely blocked IPP-3-, CDP-, TMPP-, and EDHP-induced PE in zebrafish embryos. For BPDP, we are currently finalizing BPDP-alone and BPDP+D-Mannitol exposures, albeit we expect that D-Mannitol will also block BPDP-induced PE. Overall, our findings to date suggest that chemically-induced PE may be 1) dependent on the osmolarity of exposure media and 2) driven by a disruption in osmoregulation across the embryonic epidermis. Therefore, our findings underscore the need to standardize the osmolarity of exposure media in order to minimize the potential for false positive/negative hits in zebrafish embryo-based chemical toxicity screens conducted around the world.

**1.07.P-Th053 Paired Computer-Assisted Sperm Analysis (CASA) and ATP Quantification Methods to Assess Reproductive Capacity of Male Mummichog (*Fundulus heteroclitus*)**

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While many methodologies exist to assess female reproductive competence in aquatic species, there are little to no standard methods developed for male reproductive health. Though male contribution to offspring success has historically been considered relatively small, increasing bodies of evidence support that sperm quality can have a lasting influence on the health of offspring. Current sperm quality characterization methods rely on expensive, specialized equipment that prevent widespread use of sperm quality assays when conducting reproductive health assessments of a particular system. To counter this, we developed a field-adaptable sperm motility characterization method able to be conducted with only standard laboratory equipment, validated using the male mummichog, *Fundulus heteroclitus*. Most notably, computer-assisted sperm analysis (CASA) was conducted on sperm from field-collected mummichogs and analyzed using an open-source ImageJ plugin. Because sperm movement is powered by ATP release, ATP was also quantified in inactivated sperm from the same fish using a firefly-luciferase ATP microplate assay. These methods were used on sperm from field-collected *F. heteroclitus* sourced

from two historically contaminated US rivers (Christina River, DE, USA; Anacostia River, MD, USA) and from a clean reference location (Wye River, MD, USA). These systems have a history of urban legacy contaminants, including PCBs, PAHs, and other chemicals associated with industry. Ten male mummichogs were collected from each site on each river and their sperm motility recorded in the field for subsequent CASA. Samples for ATP analysis were cryopreserved and taken to the lab for microplate analysis. Compared against the Wye River reference, all historically impacted sites showed some level of decreased ATP stores, though this varied substantially between fish at each site. Total motility and movement characteristics were marginally higher in reference fish than exposed populations and correlated well with ATP quantity, both by sites and generally by fish. This indicates that in many instances, supplementing traditional CASA with ATP quantification can be useful for explaining decreased motility, however additional analysis is required to establish if impairment is related to malformed anatomy rather than inadequate energy stores.

#### **1.07.P-Th054 Modification of the Comet Assay to Assess Sperm DNA Integrity in the Male Mummichog (*Fundulus heteroclitus*)**

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As genomics advances in popularity, greater importance in the field of toxicology is placed on DNA integrity in response to common contaminants. This is particularly true of endocrine disruption research, as abnormal gene expression can disrupt hormone signaling and other normal processes. While promising and descriptive, these methods are costly and not yet widely accessible to labs for inclusion in general reproductive health damage assessments. To combat this, the Comet assay (single-cell gel electrophoresis) done on sperm cells can be used as an informative screening tool to assess for DNA damage in a particular system using standard laboratory equipment. While traditionally done on blood and liver cells, the Comet assay can be adapted for sperm cells by decreasing standard unwinding and electrophoresis times. These methods were used on sperm from field-collected mummichogs (*Fundulus heteroclitus*) sourced from two historically contaminated US rivers (Christina River, DE, USA; Anacostia River, MD, USA) and from a clean reference location (Wye River, MD, USA). These locations have a complex history of industry and legacy contamination, as well as continued urban inputs due to proximity to cities, with prominent chemical profiles of PAHs, PCBs, and potentially associated contaminants such as zinc. Ten male adults were collected from each site on each river and their sperm cryopreserved to conduct the Comet assay in the lab. Samples were stained, imaged, and processed using an open-source Comet scoring software. As compared to the control population, all historically impacted sites displayed significantly increased levels of DNA damage as measured by tail length and intensity, tail moment, and % DNA in the tail. Extent of the damage observed also correlated well with the most contaminated sites. Most severe damage was seen in the PAH-laden Hershey Run site on the Christina River, though the Anacostia had consistently high levels of damage at the Lower Beaverdam site, as well. While the reference population had little to no damage in any fish, another site on the Anacostia (Bladensburg) had variable levels of damage, with some severe and some nearly unimpacted fish. These results indicate the continued relevance and importance of the Comet assay for detecting basic DNA damage and its usefulness in complement with other sperm quality characterization techniques to fully understand the scope of damage in a particular system.

#### **1.07.P-Th055 Pharmaceuticals and mixtures in the environment: a review on the use of zebrafish for neurotoxicity**

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The presence of neurotoxic chemical substances in the environment is an issue for both human and environmental health. Moreover, the interaction between different compounds mixed together is also a problem that can generate unpredictable effects and magnify the toxicity of the single chemicals. In this work we aim to summarise the use of zebrafish (*Danio rerio*) as a model for detecting the toxic effects of chemical mixtures. We decided to include in particular all the papers that between 2012 and 2023 focused on neurotoxicity, a widespread Mode of Action for many substances, through a behavioural approach.

This work was articulated in several steps: first, we elaborated a search string, then, we performed the bibliographic research on Embase. All the papers that resulted from the research were then analysed to determine if they met the criteria for inclusion in the final paper. All papers included were then summarised, statistical data was extrapolated and the results were reported.

The research, performed in September 2023, initially identified 779 papers that were later reduced to 171 by title and abstract reading. A screening based on the full-text reading furtherly reduced the final number to 101. All papers included were then grouped together and summarised based on the classes of chemicals involved. A statistical analysis was then performed, highlighting a growing interest for the zebrafish model for this kind of application, a prevalence of short-term exposures and a balanced use of all developmental stages. This work also shows the use of a wide variety of different assays based on different senses, on the chemicals used and the developmental stages involved.

In conclusion zebrafish, that has already known a growing use in the biomedical research over the years, is proving to be an useful tool for the detection of the overall toxicity of simple and complex mixtures. As summarised in this work, there are numerous possibilities of applications with both zebrafish adults and embryos for neurotoxicity. The use of many different assays to evaluate the same endpoints however might show the need for a more standardized approach. A more widespread use

of the early developmental stages over the adult individuals should also be preferred when possible, in line with the current “3R” principle of animal experimentation.

#### **1.07.P-Th056 Potential Triggering Effect of BPS for Diabetes by Dietary Condition in Zebrafish**

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The escalating global prevalence of diabetes has been a significant societal and economic concern. Environmental factors, particularly chemicals suspected to disrupt metabolism, have drawn attention as potential player in the etiology of diabetes. Bisphenol S (BPS) is suspected as chemical risk factor that may be associated with type 2 diabetes in human populations. This study employs zebrafish (*Danio rerio*) as a model organism to elucidate the potential mechanisms by which exposure to BPS may impact the development of T2D, especially under different dietary conditions.

To this end, 6-8 months old male zebrafish were exposed to two different concentrations of BPS for 14 days, i.e., 0.013mM (labeled as ‘BH’), and 0.004mM (‘BL’), with different dietary conditions, i.e. normal fat diet with Artemia (labeled as ‘NF’) group and high fat diet (‘HF’) group with additional egg yolk powder. Plasma insulin and glucose levels were measured, along with the transcription of genes involved in diabetes pathway, insulin signaling, and glucose metabolism.

Following exposure, NF+BH group exhibited higher fasting glucose and insulin compared to NF, supporting that bisphenol S has a potential to develop diabetes in adult zebrafish. Significantly higher glucose was observed in HF+BPS group. This observation indicates that high fat diet may increase susceptibility of zebrafish to bisphenol S toward development of diabetes. The genes associated with both the insulin signaling pathway and the diabetes pathway (*insra, insrb, pik3ca, irs1*) exhibited general pattern of upregulation when dietary condition changed to more fat or chemical dose increased. Moreover, expression of genes related to glucose metabolism (*g6pca.1, gcgra, gcgrb, hk2*) was also disrupted, implying potential influence of BPS on glucose homeostasis.

The results of this study shows possible interaction of dietary condition in the diabetogenic effects of bisphenol S in zebrafish. Investigation to unravel the role of bisphenol S in etiology of diabetes should include intricate influence of dietary effects. Further study is warranted to screen similar effects for bisphenol A analogues.

This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through "Core Technology Development Project for Environmental Diseases Prevention and Management", funded by Korea Ministry of Environment (MOE) (RS-2022-KE002173).

#### **1.07.P-Th057 Subcellular effects of an antineoplastic agent and a nanomaterial to zebrafish**

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Emerging contaminants (ECs) ECs interact with various compounds in aquatic environments, posing threats to ecosystems. Traditional hazard and risk assessment procedures often focus on individual compounds, but in the environment, organisms face intricate mixtures of stressors, highlighting the need for a comprehensive understanding. ECs, such as antineoplastics agents (AAs) and engineered nanomaterials (ENMs), present significant challenges in environmental risk assessment due to their limited regulation and understudied impacts. AAs, categorized as micropollutants, exhibit cytotoxicity at low concentrations, posing potential threats to aquatic organisms. Layered double hydroxides (LDHs) are stimuli-responsive nanomaterials with diverse applications, including as nanocarriers of drugs like AAs. This highlights the need for a comprehensive understanding of their combined effects, particularly at the subcellular level. Thus, this study aims to assess the biochemical and genotoxic joint effects of Mg-Al LDH and epirubicin in *Danio rerio*.

*Danio rerio* embryos, a well-established model organism, were exposed to no-effect concentrations of Epirubicin and Mg-Al LDH, in single and mixture exposures, according to the OECD 236 FET protocol. For the mixture exposure, a full factorial design was applied. DNA damage (evaluated through the comet assay) was assessed after 96 hours of exposure, while neurotoxicity and oxidative stress-related biomarkers were examined at the end of the 120-hour exposure period.

Epirubicin induced 15 – 25 % of DNA damage, as determined by the analysis of tail DNA, in zebrafish exposed to concentrations higher than 132 µg/L. The DNA damage caused by Mg-Al LDH was below 10 %. Epirubicin caused no neurotoxic or oxidative stress effects on *D. rerio* larvae at concentrations of up to 670 µg/L. No oxidative stress was observed after exposure to Mg-Al LDH, but larvae exhibited significant AChE activity inhibition at concentrations above 20 mg/L of Mg-Al LDH. These findings provide valuable insights of the subcellular effects promoted by mixtures of emerging compounds, like AAs and ENMs, in aquatic organisms and may assist in their future environmental hazard and risk assessment.

#### **1.07.P-Th058 The impact of nano-polypropylene accumulation in the brain on behavioral changes in Zebrafish (*Danio rerio*)**

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Plastic pollution in the environment has emerged as a new contaminant with potential toxicological impacts on various organisms. Among them, nanoplastic, which has a small size and high bioavailability, and exist anywhere in the environment, poses a great threat to the ecosystem. Although research on the toxicity of nanoplastic is actively ongoing, our understanding remains limited. In this study, toxicity experiments were conducted using directly synthesized nano-polypropylene (PPNPs). The accumulation of PPNPs in the brina was confirmed, and the neurotoxic effects of accumulated PPNPs were investigated. Zebrafish (*Danio rerio*) were chosen as the experimental model due to their genetic and biological similarity to humans and comparable physiological characteristics with other animal models. Zebrafish exposed to four concentrations of PPNPs under optimal rearing conditions were used for analysis. The presence of PPNPs in the zebrafish brain was confirmed through pyrolysis gas chromatography-mass spectrometry and bio-transmission electron microscope. Behavioral monitoring assessed mobility and aggression, while enzyme levels of neurotransmitters and neural activity were measured. The accumulation of PPNPs in the brain, verified by TEM micrography, led to neurotoxicity, resulting in reduced mobility and aggressiveness. Changes in neurotransmitter levels and neural activity, associated with behavior further supported these findings. In conclusion, this study suggests that environmental plastic pollution can induce neurotoxicity in organisms, emphasizes the need for appropriate management of these materials.

#### **1.07.P-Th059 Toxicity assessments of benzophenone-related derivatives in medaka fish**

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Benzophenones (BPs) are a category of chemicals with similar structures, commonly used as UV filters (e.g., BP itself and BP1-BP12) in personal care products such as sunscreens, skincare products, and body washes. Other BP-derivatives are often used as photoinitiators, binders, or additives in printing inks of food contact materials. Studies have shown frequent occurrence of certain BPs (as UV filters) in various environmental matrices and organisms. In particular, BP3 increases coral bleaching rates and exhibits estrogenic activity in aquatic life. However, much less studies report on ecotoxicity of 10 BP-derivatives. This study aims to analyze *in vivo* toxic effects of specific BPs-derivatives on medaka (*Oryzias latipes*) fish. The LC50 value from 96 hr-acute mortality of 7-day post hatching medaka larvae showed that DEAB was the most toxic compound and BP was the least toxic compound. With the 7 day-sublethal exposure, 4BPB and DEAB dose dependently increased larval mobility and oxidative stress responses at tested concentrations. We will further investigate the toxic mechanism of 4BPB and DEAB in medaka fish regarding fish behavior under environmentally relevant exposure conditions.

#### **1.07.P-Th060 Transcriptomic alterations caused by progestogens during early zebrafish development**

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Evidence has shown that endocrine disruption chemicals (EDCs) can alter reproductive aspects in fish. These alterations affect their reproductive status, putting the continuity of the populations at risk and impacting biodiversity conservation. In the past decades, the effects of EDCs on fish reproduction have been the focus of increasing research. However, only a few studies have addressed their effects before pubertal onset and little is known about their precise mode of action. Synthetic progestagens and their derivatives, widely used for pregnancy maintenance in humans or as growth promoters in livestock, have proven ecotoxicological effects and appear as those with the highest environmental concern after synthetic estrogens such as ethinyl estradiol (EE2). It is well known that progestagens have a direct effect on the onset of meiosis and are considered key players in this process, however, the molecular mechanism by which they can alter puberty in fish still remains elusive. The purpose of our study was to determine the effects of progestagens, previously shown to alter steroidogenic genes at early zebrafish development stages, at the transcriptome and lipidome levels. Based on already published studies we first exposed embryos to 0.6 nM of progesterone (P4), levonorgestrel (LNG), and mifepristone (RU486) from 0 to 5 days post-fertilization (dpf). Results from RNA sequencing showed that exposure to this concentration did not provoke major alterations in the transcriptomic profile of fish. Therefore, a second exposure following a dose-response approach was performed exposing embryos to 0.2 nM - 16.2 nM of progesterone (P4), levonorgestrel (LNG), and mifepristone (RU486) from 0 to 5 days post-fertilization. At present, we are characterizing the transcriptomic response of embryos exposed to the progestogens by real-time RT-PCR. In the near future, we aim to perform lipidomic analysis by an untargeted liquid chromatography-high resolution mass spectrometry (LC-HRMS). Consecutively, we aim to study the long-term consequences of exposure to progestins during early development (0-5 dpf) by performing transcriptomic and lipidomic analyses in juvenile zebrafish to a better understanding of the modes of action of progestins, which leads to better protection of environmental health. The research leading to these results has received funding from the Spanish Ministry of Science and Innovation MCIN/AEI/10.13039/501100011033 (Grant PID2021-122929OB-C33).

#### **1.07.P-Th061 Translocation and Toxicity of Additional Chemical Substances in Expanded Polystyrene Microplastics on Black Rockfish *Sebastes schlegelii***

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Republic of

Expanded polystyrene (EPS) has been manufactured by adding hexabromocyclododecane (HBCD), an organic flame retardant, but has been regulated due to the harmfulness and environmental problems caused by HBCD. However, numerous fragments originating from EPS products manufactured and recycled in the past are ubiquitous in the marine environment, and this study confirmed the biological effects of EPS-derived large MP (EPS-LMP) and added HBCD on black rockfish (*Sebastes schlegeli*), a commercially important fish species widely distributed along the coast of the South Korea. EPS-MP was made of 4.5 mm beads and 2 mm fragments and administered orally using gelatin capsules to determine toxic effects after 4 and 7 days. As a result, no deaths occurred during the 7 days of exposure, and no effects on growth were confirmed, but EPS-MP still existed in the gastrointestinal tract. Intrahepatic HBCD was detected in a concentration-dependent manner, and negative effects on cytotoxicity on leukocytes and intrahepatocyte toxicity indicators were confirmed in groups exposed to 100 mg of LMP. The exposure response and toxicity mechanism of black rockfish according to changes in size or concentration of EPS-LMP were verified through a multi-omics approach that analyzed transcriptome and metabolic changes. Our findings highlight the importance of the toxic effects of translocated MP in resident species in understanding the toxic effects through microplastic contamination.

#### 1.07.P-Th062 Zebrafish as a Model for Screening Chemicals Linked to Preeclampsia Induction

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Preeclampsia is one of the pregnancy-related diseases accompanied by hypertension and proteinuria. In cases of early onset or severity, preeclampsia can lead to adverse outcomes such as premature placental abruption, miscarriage, and fetal death.

Research on the etiology and adverse outcome pathways (AOPs) of pregnancy-related diseases is essential to prepare for the health of future generations and establish a precision public health. In this study, we explored the usefulness of zebrafish as an animal model to screen for chemicals associated with preeclampsia induction. A literature review was performed to identify AOPs in preeclampsia and to investigate molecular initiating events (MIEs) and key events (KEs). Among these, we explored biomarkers available in zebrafish, focusing soluble fms-like tyrosine kinase-1 (sFLT-1), soluble endoglin (sEng), vascular endothelial growth factor (VEGF), placental growth factor (PIGF), and transforming growth factor- $\beta$  (TGF- $\beta$ ), which are used as angiogenic indicators, and investigated existing studies related to exposure to environmental pollutants. Preeclampsia is estimated to have heritability of approximately 55%, and is caused by imbalanced maternal nutritional status, lack of vitamins and minerals, and exposure to environmental pollutants (e.g., cadmium, bisphenol A, phthalates, flame retardants, and perfluorinated compounds). Impaired trophoblast invasion and spiral artery remodeling, placental ischemia and hypoxia, and changes in the renin-angiotensin-aldosterone system (RAAS) were identified as the MIEs and KEs of preeclampsia. Although zebrafish does not have a placenta, they express genes and proteins (e.g., vegfa, sflt-1, and eng) related to the mechanism of preeclampsia. The results of this study indicate that zebrafish possesses genes and proteins related to the preeclampsia, and can be used as an animal model to screen for chemicals related to causing preeclampsia.

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Keywords: adverse outcome pathway, angiogenesis, biomarker, preeclampsia, zebrafish

#### 1.07.P-Th063 Effects of Differently Aged Tire-Wear Particle Suspensions on *Danio rerio*

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Tire wear particles (TWP) are formed when friction occurs between tires and the road surface. Adding to the physical damage that these TWP may cause to organisms, a simultaneous toxicity may be induced by the chemical compounds that constitute the TWP and are released over time into the environment. Thus, this study aimed to evaluate the toxicity of a TWP suspension at environmentally relevant concentrations (between 0.5 and 512 mg TWP/L), considering three ageing stages of the suspension: immediately after preparation, 7, and 30 days after preparation (0dA, 7dA, and 30dA, respectively), using *Danio rerio* as a model species. Due to the homology between this model and humans, inferences can also be made about the potential effects that TWP can cause in humans. The 96-h Fish Embryo Toxicity (FET) assays followed the OECD guideline 236 (OECD, 2013) using two life stage stages (embryos and post-hatching larvae) to determine potential different routes of uptake due to the occurrence of key events prior and post-hatching, such as eye and retina formation (in embryos) and protruding mouth development in larvae. Mortality and malformations, in addition to the determination of the heartbeat rate (HBR), total and interocular length (TL and IL, respectively), swim bladder area (SBA), and behaviour (total swim distance and time) were registered.

No significant mortality and malformations were registered in all assays with embryos and larvae (>10%). In embryos, HBR presented a pattern of tachycardia between concentrations of 0.5 and 8 mg TWP/L, whilst a pattern of bradycardia at higher concentrations (<32 mg TWP/L), for all ageing stages. The IL was significantly higher ( $p<0.05$ ) in organisms exposed to tire wear particles concentrations equal to or higher than 8 mg/L in a non-aged particle suspension, whilst significantly higher in organisms exposed at the concentrations 0.5, 2, and 8 mg/L of a tire wear particle suspension aged for 7 days, with effects tending to disappear over time. In the post-hatching larvae, SBA decreased significantly ( $p<0.05$ ) at all tested concentrations (except 0.5 mg TWP/L) compared to the control\_0dA. The activity time of larvae from the embryo assay was close to zero. The results obtained highlight that further studies are needed to increase knowledge on TWP effects on understanding the impact that this type of particulate may have on ecosystems and, ultimately, human health.

## **1.08.P In Silico Ecotoxicology: Using Existing and Emerging Data to Characterize Chemical Impacts from Individuals to Ecosystems**

### **1.08.P-Mo049 Assessing Chronic Effects of Chemical Pollution on Biodiversity Using Mean Species Abundance Relationships**

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Chemical pollution is a persistent threat to freshwater ecosystems and biodiversity. The long-term impact of chemical pollutants on our natural environments is a growing challenge requiring innovative methodologies to understand better and address the ecological risks associated with such pollutants. This research addresses the need for a predictive assessment of the long-term impacts of chemical pollution on biodiversity. We utilized the Mean Species Abundance Relationships (MSAR) methodology as a biodiversity measurement tool. This approach can provide the Mean Species Abundance as a function over the concentration of a chemical, allowing the interpretation of the impact of a chemical on biodiversity. This study aims to provide a tool to predict chronic Mean Species Abundance Relationships based on acute values.

The longer the exposure to a chemical, the less concentration is needed to result in a lethal effect. The resulting time-dependency can be described with the median Lethal Concentration (LC50). This method is based on the Critical Body Residue concept and, more precisely, the Lethal Body Burden approach and can be extended to other LC50-based tools. By fitting acute LC50 values to the time-dependent equation, LC50 values for any time point can be estimated and used to derive the Mean Species Abundance Relationships. Data from a case study with six freshwater arthropods exposed to imidacloprid was used to test the methodology for two different time points (days 21 and 28). Additionally, it was tested if chronic data is necessary to obtain a reasonable fit or if acute data is enough by including chronic values in the fitting up to the time point to be predicted. The chronic data was then used to validate the LC50 fits and MSAR.

For predicting LC50 values, the model overestimates the acute toxicity and underestimates the chronic toxicity of imidacloprid for certain species. This can be caused by the biotransformation of imidacloprid to the likewise toxic imidacloprid-olefin. However, the estimations of the chronic Mean Species Abundance Relationships are similar to the measured chronic MSAR. If only acute data were used for the estimation, the mean difference in Mean Species Abundance (MSA) between the prediction and the fitted MSAR is 6%. If chronic data was included, this difference lowered to 3% for day 21 and 2% for day 28.

### **1.08.P-Mo050 Integrating Time-Resolved Gene-Expression Data into TKTD Models to Approximate Toxicodynamic Damage**

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The immense productivity of the chemical industry requires an improved predictive risk assessment that can handle constantly evolving challenges. Integrating OMICs data into mechanistic models offers a promising solution by linking cellular processes triggered after chemical exposure with observed effects in the organism. The envisioned outcome of such approaches is the prediction of chemical effects for untested species–substance combinations and mixtures based on molecular data. However, its success hinges on the quantitative description of the temporal dynamics of molecular markers in response to chemical exposure.

The transcription factor *nuclear factor erythroid 2-related factor 2* (*Nrf2*) has been identified as a master regulator of the detoxification process and is tightly linked to chemical and oxidative stress in organisms. This makes *Nrf2* an optimal candidate for approximating the dynamic damage state in toxicokinetic-toxicodynamic (TKTD) models, thereby enabling the integration of OMICs data into mechanistic models.

In this work, we develop and apply a biologically anchored TKTD model, which describes the key processes in the *Nrf2* signalling pathway, linking the gene expression level of *nrf2* to detoxification and lethality. The model was fitted on time series of internal concentrations, *nrf2* gene-expression and lethality observed in zebrafish embryos exposed to diuron, diclofenac, and naproxen in over 200 experiments, using a Bayesian approach.



*Nrf2*-expression proved to be a reliable predictor of lethality for naproxen and diuron, accurately predicting lethality even in data-limited concentration ranges. In contrast, *nrf2*-expression was no good predictor for lethality under diclofenac exposure, possibly due to a differential expression pathway. By using *nrf2* as a predictor for lethality, highly similar lethality-related model parameters were obtained for different chemicals, opening the door for cross-substance extrapolation.

This study shows that *nrf2*, as a master regulator of the chemical and oxidative stress response, can serve as a proxy for lethality after exposure to different substances. Our findings highlight the advantage of the OMICs-TKTD integration in discerning stress response pathways based on the temporal dynamics in the molecular stress response. Finally, the work demonstrates the feasibility of integrating dynamic OMICs data into established TKTD models for approaching the goal of quantitative toxicity prediction across compounds.

#### **1.08.P-Mo051 Forwarding maturation of Species Sensitivity Distributions using Machine Learning**

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The characterization of impacts of chemical pollution in ecosystems is amongst others performed by a combination of exposure data with insights from Species Sensitivity Distributions (SSDs). SSDs are the statistical description of patterns in observed ecotoxicity endpoint metrics, such as NOECs, EC50s and LC50s. Despite the versatile application of this approach, we do not fully use available collections of ecotoxicity data to generate SSDs. This presentation ventures into a Machine Learning (ML) based additional approach to the derivation of SSDs from currently available data, and presents a suite of high-utility applications of outcomes of such SSDs derived by ML.

SSD models have different uses, classically ranging from the earliest uses in the derivation of protective, regulatory environmental quality standards and the characterization of the degree of harm caused by unintended ambient mixtures. Environmental concerns have resulted in vast collections of ecotoxicity test data, such as ECOTOX and REACH-database. Despite the wealth of information on chemical hazards there are still severe data gaps for many chemicals and the already available data has not yet been extensively used to identify the sensitivity patterns regarding chemicals and species in the broadest sense of the word. In our innovative versatile approach, we investigate the hidden patterns in these available ecotoxicity data sources and how these patterns can be applied to a wide variety of use cases. For this purpose, we use Machine Learning (ML) in the heart of the employed methods, to address, and potentially solve, the issue of data gaps. Particularly, the current data gaps are hampering the ecotoxicity assessments and are amongst the most pressing problems that limit the practical use of techniques such as Safe and Sustainable by Design.

Here, we aim to present the outputs of some example analyses, with the aim to highlight how we can derive SSDs based on both measured and ML-predicted ecotoxicity metrics. We show not only that we can derive SSDs for vastly more compounds based on existing data and underlying patterns, but also how those SSDs can be employed for at least three main purposes in environmental protection, assessment and management. We also show whether, how and to which extent the newly derived SSDs can be trusted as tools for practical purposes.

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#### **1.08.P-Mo052 The Effects of Pollution and Foraging Adaptation on the Stability of Ecological Communities**

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The relationship between the adaptive responses of predators and the stability of communities has been previously analyzed only considering pristine environments, even though many ecological systems are exposed to pollutants, among other disturbances. Many pollutants tend to accumulate in the tissues of organisms, resulting in species present in trophic webs not only transmitting their effects among themselves, but also transmitting the pollutants through their trophic interactions. In this context, adaptive behavior, considered in this study as changes in prey preference, can inhibit pollutant transport through trophic networks without completely limiting biomass flow. In this study, the effectiveness of adaptive responses of predators in promoting the persistence and diversity of species in trophic networks subjected to stress from a bioaccumulative pollutant was evaluated. A community model based on the bioenergetic model was used, where the effects of the network topology (species richness and connectance), the pollutant concentration and the adaptive behavior over the species persistence and Shannon-Wiener diversity index were distinguished. In this work it was shown that adaptive behavior promotes species persistence and diversity in communities subject to stress from a bioaccumulative pollutant. Therefore, the adaptive responses of predators have a beneficial role on the stability of communities affected by bioaccumulative pollutants.

#### **1.08.P-Mo053 Vitamin K 2,3-epoxide reductase molecular identity range the binding affinities to anticoagulant rodenticides of Rodentia**

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Rodents are notorious for being one of the pests with the greatest impact on agricultural production and public health. Anticoagulant rodenticides (ARs) are commonly used to control rodent populations by inhibiting the vitamin K 2,3-epoxide reductase (VKORC1) enzyme, which leads to internal haemorrhages and finally death of the animal. Docking analysis previously demonstrated that human VKORC1 variation is associated to anticoagulant resistance and affects binding interfaces (2). In rodents, research has been focused on the main pest species, such as the mouse, black rat, and grey rat. However, little is known about the influence of AR in other rodent species, despite the importance of this phylum in wild and agro-ecosystems. The hypothesis of this work is that rodent phylogenetic and structural differences among VKORC1 sequences do not range the binding properties of AR. To test this null hypothesis, we searched for complete VKORC1 sequences of different rodents in public databases and characterized the binding predictions by computational analysis. Rodentia phylum has differential sensitivity to second generation AR depending on species and families. The range of Rodentia sensitivity was (from highest to lowest) flocoumafen, difenacoum, brodifacoum, bromadiolone and difethialone. Long-tailed chinchilla is the most sensitive rodent to flocoumafen, difenacoum and brodifacoum, which bind very strongly to its enzyme (13 Kcal/mol). Guinea pig and Lesser Egyptian jerboa were also very sensitive to flocoumafen and difenacoum as binding affinities were above 12 Kcal/mol. Difenacoum has binding affinities higher than 14 Kcal/mol in two Muridae and one Cricetidae. Among Cricetidae family, European snow vole had binding affinities higher than 14 Kcal/mol to brodifacoum. Specific sensitivities could be used to either control populations in case of rodent pests or to forbid the use of AR in ecosystems with protected species. The present work is a powerful *in silico* tool for pest control companies and biocides regulatory agencies.

#### **1.08.P-Mo054 Using Comparative Genomics to Develop ‘Digital Twins’ to Support Ecotoxicological Predictions**

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Our rapidly changing world is placing ecosystems under unprecedented pressure, including exposure to a wide range of chemical toxicants. Given the limited ability to test the impact of these toxicants across all species, it becomes crucial to unravel the intricate biological cascades that influence how different non-model organisms respond to such pollutants. By generating *in silico* representations of organisms and harnessing genomic data, this project seeks to reveal key characteristics underlying comparative ecotoxicological responses. From this we can predict species sensitivities to different toxicants, thereby protecting species critical for ecosystem processes or of high conservation value. We have developed an automated pipeline to streamline the process of quality assessment, pre-processing, *de novo* assembly, and annotation of publicly available transcriptomic data. This pipeline is optimised to operate within a Slurm-based system designed for high-performance computing (HPC) infrastructures and will be made available *via* GitHub. The transcriptome annotations are mapped to enzymes underpinning phase I metabolism to provide evidence to support relative Toxicokinetics (TK) and onto Adverse Outcome Pathways (AOPs), focusing on Molecular Initiating Events (MIE) and Key Events (KE) identified in eukaryotic organisms, to provide mechanistic insight into comparative Toxicodynamics (TD).

The pipeline has been deployed and evaluated against a diverse array of organisms to investigate the impact of laboratory vs wild population (*Enchytraeus albidus*), to compare *de novo* transcriptome vs genome (*Acyrtosiphon pisum* vs *Aphis fabae*) and to investigate organisms with large genomes with known transcript redundancy (*Porcellio scaber* – genome size ~5.7 Gbp). To augment this methodology, we are developing pipelines for analysing target receptor ortholog conservation across different species alongside applying *de novo*-based molecular docking techniques in evaluating receptor-ligand interactions. Further, we aim to transition these pipelines to a Nextflow framework to offer improved efficiency across heterogeneous HPC hardware. In conclusion, this study will develop a comprehensive automated data platform incorporating aspects of TK and TD and use mechanistically informed approaches to support AOP-based species sensitivities distributions (SSD) predictions.

#### **1.08.P-Mo055 ADORE is for Lovers: A Benchmark Dataset for Machine Learning in Ecotoxicology**

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The use of machine learning for predicting ecotoxicological outcomes is promising, but underutilized. The curation of data with informative features requires both expertise in machine learning as well as a strong biological and ecotoxicological background, which we consider a barrier of entry for this kind of research. Additionally, model performances can only be compared across studies when the same dataset, cleaning, and splittings were used. Therefore, we provide ADORE, an extensive and well-described dataset on acute aquatic toxicity in three relevant taxonomic groups (fish, crustaceans, and algae). The core dataset describes ecotoxicological experiments and is expanded with phylogenetic and species-specific data on the species as well as chemical properties and molecular representations. The dataset spans Apart from challenging other researchers to try and achieve the best model performances across the whole dataset, we propose specific relevant challenges on subsets of the data and include datasets and splittings corresponding to each of these challenges as well as in-depth characterization and discussion of train-test splitting approaches and their relevance for this use-case.

#### **1.08.P-Mo056 Identifying Molecular Endpoints to Assess the Biological Response of Contaminant of Emerging Concern in Marine Mammals: an In Silico Approach**

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Due to their ecology marine mammals accumulate some of the highest levels of environmental contaminants of all wildlife. Contaminants of emerging concern (CECs) enter the environment from various anthropogenic sources and are distributed throughout environmental matrices. They have been detected in water at concentrations significantly higher than expected and their risk to human and environmental health may not be fully understood yet. CECs comprise a vast array of contaminants: pharmaceuticals such as personal care products (PPCPs), nonsteroidal anti-inflammatory drugs (NSAIDs), cardiovascular system drug (CVSD), steroidal hormones and antibiotics; Endocrine Disrupting Chemicals (EDCs) such as bisphenol A (BPA) phthalate esters, perfluoroalkyl and polyfluoroalkyl substances (PFAS) which are commonly spread in marine organisms ranging from benthic invertebrates up to top predators. The toxicological data of emerging contaminants on marine mammals is still scarce for several compounds and the toxicological impact of these contaminants needs further assessment. In silico models are a promising alternative to in vitro and in vivo methods to predict the toxicological effects of chemicals on mammalian model organisms (e.g. rat, human). These approaches continue to grow in capability and applicability to predictive toxicology. Numerous databases such as ChEMBL, EMBL-EBI and ECOTOX could be used as a comprehensive knowledge base providing data on the environmental toxicity of these chemicals on aquatic and terrestrial species. The aim of this study was to utilize in silico approaches such as Similarity Ensemble Approach (SEA), SwissADME and admetSAR, which are some of the most used in silico tools, in conjunction with literature research to identify specific endpoints strengthening our knowledge and our understanding of the biological effect of the CECs. The identified endpoints will be tested ex vivo (e.g. tissues/biopsies) on charismatic species such as: *Balaenoptera physalus*, *Stenella coeruleoalba* using Digital PCR assay (ddPCR).

#### **1.08.P-Mo057 Developing an Ecosystem-Level Assessment Framework for Ecological Impacts of Accidental Chemical Exposure in Korean Freshwater: A Simulation-Based Approach Using the AQUATOX Model**

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Recent ecological risk assessments (ERAs) encompass evaluations from the cellular to ecosystem levels. However, higher-level assessments, particularly at the ecosystem level, are often resource-intensive in terms of labor, cost, and time. Consequently, ecologists and environmental assessors are increasingly turning to modeling approaches. The United States Environmental Protection Agency (US EPA) has developed the AQUATOX model, an ecosystem-level risk assessment model designed to evaluate the effects of disturbances on freshwater environments, including chemical exposure, environmental changes, and ecological disruptions.

This study aims to conduct AQUATOX simulations and develop a library specifically tailored to the Korean freshwater system, with a focus on simulating the effects of phenol exposure. The target region for this study is the rivers in Korea. The Korean Ministry of Environment routinely investigates the ecological composition, phenol concentrations, and water quality of freshwater systems, periodically publishing the findings on the Water Environment Information System (WEIS, <http://water.nier.go.kr/web>). The model system is constructed using a substantial dataset of ecological information gathered from Korean rivers, considering the analyzed interactions between organisms in the food web of Korean freshwater and the ecological characteristics of domestic species in Korean rivers.

Monitored data covering the period from 2008 to 2020 from the WEIS system is utilized in model construction. The resulting model system and built library are validated using ecological monitoring data from regions exposed to phenol. The outcomes of this study will contribute to the development of a chemical assessment system for Korean rivers, utilizing an ecological model approach.

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#### **1.08.P-Mo058 Derivation of environmental quality standards for free cyanide in freshwater and marine surface waters by species sensitivity distribution including censored data**

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The currently available Dutch environmental quality standards (EQSs) for free cyanide and cyanide complexes are regularly debated and considered to be outdated. Hence, there is an urgent need to update the EQSs for cyanide. The aim of the present study was therefore to derive new maximum acceptable concentrations (MAC) and annual average (AA) EQSs for free cyanide, following the 2018 version of the Technical Guidance for Deriving Environmental Quality Standards of the Water Framework Directive (WFD). To this end, ecotoxicity data were collected from previous national and international EQS derivations, supplemented with a literature search to gather additional ecotoxicity data generated since the publication of these reports. All collected ecotoxicity data were evaluated for reliability and compliance with strict standards, such as analytical verification. Data considered reliable were used for the derivation of the EQS values. The pooled freshwater and marine, acute

ecotoxicity data set consisted of reliable results for 35 species, distributed over 8 taxonomic groups. The pooled chronic ecotoxicity data set consisted of results for 13 species, distributed over 7 taxonomic groups. As both the acute and chronic dataset met the WFD criteria for deriving a species sensitivity distribution (SSD), a probabilistic approach was taken. However, the datasets include several censored data which are traditionally excluded when deriving an SSD. In the present paper, we present a solution for the censored data points using the R-package ETX 3.0-132, that provides functionality for fitting univariate SSD-related distributions to possibly censored toxicity data using a Bayesian simulation technique. The HC5 values derived from these acute and chronic SSDs were 17 and 0.66 µg CN-/L, respectively. Applying a default assessment factor of 10 to the HC5 from the acute SSD resulted in a MAC-EQS of 1.7 µg CN-/L for both freshwater and marine surface water. Applying assessment factors of 3 and 5 to the HC5 of the chronic SSD resulted in AA-EQSs of 0.22 µg CN-/L for freshwater and 0.044 µg CN-/L for marine surface waters, respectively. These MAC-EQS and AA-EQS values are generally in line with derivations by other (inter)national water authorities, but the present environmental quality standards have been derived in full accordance with the WFD guidance, using only reliable data and including censored values.

#### **1.08.P-Mo059 Developing a Database for Comprehensive Assessment of Fish Reproductive Toxicity**

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Traditional animal-based risk assessment of chemicals is struggling with the demands of 21<sup>st</sup> century safe and sustainable chemical development. In the project SAFE: Innovative Safety Assessment of Fish Adverse Effects, we are working to replace some of the costliest and most time-consuming tests in environmental toxicology - reproductive toxicity of chemicals to fish. Our approach incorporates computational (knowledge networks, machine learning) and experimental methods (molecular and cellular assays using permanent fish cell cultures). One of the main challenges of the development of such alternative tests is training the model and evaluating it against animal tests. The main reason being that it is expensive (and ethically problematic) to produce high quality *in vivo* validation data, while existing data are challenging to come by and sometimes difficult to interpret. This is why the very first step in our project was to collect and curate all available *in vivo* fish reproductive studies, especially those reporting reproductive endpoints and gene expression.

We collected fish reproductive toxicity data from EnviroTox (HESI) and EcoTox (US EPA) databases, the Gene Expression Omnibus (NCBI) and systematic literature reviews. When combining the gathered data into a unified database, we encountered several challenges: reproductive endpoints reported under non-standardized names, data reported across multiple publications, use of non-standard units, use of different statistical measures (LOECs, NOECs, ECs), missing metadata and data reported only in graphical format. We standardized the diverse information curated from the available resources to develop a unified database of fish reproductive toxicity. The next steps in our vision are to test permanent cell cultures and to build machine learning models that would be able to predict reproductive toxicity based on the fish cell assays and available historical data.

#### **1.08.P-Mo060 In Silico Approaches to Characterize Chemical Impacts on Genetic Diversity**

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Population-level diversity is the bridge between individual organism and species-level effects. Within-species genetic diversity is susceptible to chemical pollution, and understanding the potential impacts of chemicals is essential to determine the resilience and adaptation potential of different species. However, of the three levels of biodiversity (genetic, species, and ecosystem), genetic diversity has been reported to receive the least attention. Field-based methods to assess genetic diversity depend on sequencing large numbers of organisms in a given population/geographic region or using environmental DNA. However, finding connections between these methods and chemical exposures is challenging, and difficult to do prospectively. Lab-based methods to assess genetic susceptibility to chemicals have also been used, but are impossible for all possible species/chemical combinations that may occur. We advocate that computational methods can be used to complement and enhance existing methods to assess chemical impacts on genetic diversity. We describe new opportunities to harness *in silico* approaches used in human health toxicology to inform genetic susceptibility to chemicals in non-human organisms. These include data integration approaches to form connections between chemicals, genetic data, and adverse outcomes in different species, and adverse outcome pathway (AOP)-based approaches. We propose that chemical pollution impacts on genetic diversity can serve as an early warning sign for more extreme adverse ecosystem effects (e.g., species loss), and thus combined environmental monitoring and computational methods can enable development of ecosystem protection and risk mitigation measures to prevent biodiversity loss from chemical pollution.

#### **1.08.PC In Silico Ecotoxicology: Using Existing and Emerging Data to Characterize Chemical Impacts from Individuals to Ecosystems**

#### **1.09.A Invertebrate Species in Ecotoxicology: Challenges and Opportunities in a Global Change Scene**

### **1.09.A.T-01 A Systematic Evidence Map of Molluscan Endocrinology and an Investigation of the Effects of a Pharmaceutical 5-alpha-Reductases Inhibitor, Dutasteride, on two Freshwater Gastropod Molluscs**

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Molluscs, integral to ecosystems as habitat engineers and food sources, face unprecedented threats from pollution. Notably, the historical impact of tributyltin (TBT) causing 'imposex' by altering the sex organs of female snails, contributed to a global decline in marine snail populations. While molluscs can serve as early indicators for endocrine-disrupting chemicals (EDCs), current testing guidelines lack mechanistic understanding due to the intricate differences in molluscan endocrinology compared to vertebrates. Molecular investigations have demonstrated that molluscan genomes lack essential enzymes to induce vertebrate steroidogenesis as well as several nuclear steroid receptors. Some steroidogenic enzymes do appear to exist in mollusc genomes, but their function remains unknown. Most notably, the enzymes 5-alpha-reductases (5 $\alpha$ R1, 5 $\alpha$ R2) that metabolise testosterone to dihydrotestosterone in vertebrates, are present in molluscs. Previous work in our lab has shown that developmental exposure of *Biomphalaria glabrata* embryos to pharmaceutical 5 $\alpha$ R disruptors (dutasteride or finasteride) results in a highly reproducible and dose-dependent disruption to development, resulting in altered shell morphology. However, the impact of dutasteride on adult gastropods remains unexplored. To fill this gap, two experimental systems are employed to investigate the effects of dutasteride on adult gastropods: a 21-day flowthrough exposure using *B. glabrata* snails and an OECD 243 static-renewal test using *Lymnaea stagnalis*, both with nominal exposure concentrations of 0, solvent control, 1 $\mu$ g/L, 3.2 $\mu$ g/L, 10 $\mu$ g/L, 32 $\mu$ g/L and 100 $\mu$ g/L Dutasteride. A systematic evidence map has also been developed to identify existing knowledge gaps in molluscan endocrine research. Findings from our systematic investigations suggest the existence of possible less intensively investigated hormonal pathways in molluscs, involving phytoosterols (i.e. plant steroids), ecdysteroids (i.e. insect steroids) and thyroid hormones. These pathways could potentially be a target of chemical disruption that could interfere with their respective hormones, hormone-metabolising enzymes and/or hormone receptors, but currently remain underexplored. Our work aims to contribute valuable insights into the physiological role of 5-alpha-reductases in molluscs and identify novel endocrinological endpoints for ecotoxicological research that could enhance existing regulatory guidelines for testing chemicals.

### **1.09.A.T-02 Exposure of freshwater gastropod *Lymnaea stagnalis* to silver nanoparticles causes chronic and transgenerational effect**

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The traditional focus of ecotoxicological studies has been primarily on a single generation. Multigenerational research in ecotoxicological testing is needed to improve predictive approaches in ecological risk assessment. Given widespread use of silver nanoparticles (AgNPs), silver nanoparticles (AgNPs) are increasingly being detected in aquatic environments.

In this study, we investigated here the transgenerational effects of AgNPs on the life traits of the freshwater model gastropod *Lymnaea stagnalis* over two generations. Reversibility of the effects using recovery experiments was also performed. The results showed that AgNPs induced high Ag bioaccumulation throughout the soft tissues of *L. stagnalis* parents after 21 days of exposure. Increased ROS production and reduced condition index and fecundity were observed after exposure to AgNPs at 500  $\mu$ g/L. These results underline that, upon exposure to AgNPs, adult gastropods may allocate more resources to resist oxidative stress rather than growth or reproduction. In addition, Ag accumulation and ROS production in egg clutches were significantly related to the duration and concentrations of parental exposure. On the other hand, exposure of single eggs, after transfer to a clean medium, similar Ag bioaccumulation and ROS production were observed from eggs whose parents had been pre-exposed to 50 and 500  $\mu$ g/L AgNPs. demonstrated that biological effects were persistent for the next generation.

The current explicit consideration of offspring performance adds value to existing toxicity testing programs. There are important effects of parental exposure duration on offspring effects, and parental exposures can lead to transgenerational changes in resistance, which have significant implications for toxicity testing and adaptive responses. These transgenerational effects suggest the need for multigenerational testing to assess the environmental risks of MNPs in aquatic organisms.

### **1.09.A.T-03 Tracking Temporal and Spatial Ecotoxicological Effects of Suspended Particulate Matter in Lake Geneva**

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In aquatic environments, suspended particulate matter (SPM) constitutes an important interface between water and sediment and hence interacts with chemicals including toxic contaminants (adsorption, transport...). In contrast to bottom sediments, SPM reacts quickly to the surrounding conditions and can show temporal and spatial fluctuations. Here, we aim to study the ecotoxicological quality of SPM collected in Lake Geneva over different seasons and months. We investigated if SPM induced effects on two model invertebrates, the benthic insect *Chironomus riparius*, and the epibenthic ostracod *Heterocypris incongruens*. We assessed key biological parameters (mortality, growth and emergence) and explored the use of molecular and enzymatic biomarkers for a deeper interpretation. Lake SPM was trapped during two successive periods of 4.5 months in Spring to Fall 2021 and 2023. In 2023, additional traps were deployed to recover monthly SPM samples at 4 different depths: 10, 30, 50 and 100 m. The quality of SPM was characterized by physico-chemical analyses (grain-size, total organic carbon, S, N and P content and metals, including total Hg). SPM samples were also submitted to targeted GC-MSMS and non-targeted LC-HRMS screening. SPM-exposed larvae were digested for analysis of potentially bioaccumulated metals. In 2021, the emergence success of *C. riparius* was significantly impacted by the second season sample, while we noticed high larval

mortality during the exposure of the first season sample. In 2023, in the ostracod bioassay, the mortality rate varied over the months and seasons but also with depth. Over 1,000 compounds were detected by LC-HRMS. Even though more substances were detected in Feb-July 2021 (172) than July-Nov 2021 (147), a large proportion of substances were detected in both periods, suggesting a clear anthropogenic impact of urban, industrial and recreational activities that contribute to the presence of micropollutants linked to lake SPM. Measured concentrations of targeted pollutants however rarely exceeded sediment quality criteria. We showed that bioassays could be used to assess the ecotoxicological quality of SPM that was dynamic over time and space. Overall, we observed good agreement among toxicity tests for a same sampling and among tests for the different sampling strategy among years. In the future, effect-directed analyses could help to identify the natural or anthropogenic pollutants responsible for the observed ecotoxicity.

#### **1.09.A.T-04 Chemical Characterization and Toxicity Assessment of Antifouling Coatings' Lixiviate: Implications for Biofouling Management**

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Antifouling coatings are commonly applied to minimize the growth of organisms on submerged artificial hard substrates. Biocide-based coatings (BC) are the most widespread ones and, currently, copper is the main biocide used in combination with other booster biocides. However, there is uncertainty regarding their performance and potential indirect environmental impact. Thus, new non-toxic coatings, such as foul-release coatings (FR), are being developed as a sustainable alternative. This study aimed to understand the effects of environmental conditions on the metal leaching from the coatings and to develop a screening procedure to assess their toxicity on selected marine species.

PVC panels with a standardized surface were coated, weighted and submerged in glass containers with 1L filtered sea water. Containers were placed in an incubator with an integrated shaker for 24h. Metal release was measured under 6 experimental conditions: 3 temperature scenarios (14, 20 and 30 °C) at 33.5 PSU; 3 salinity scenarios (20, 33.5 and 37.5 PSU) at 20°C. Additionally, uncoated PVC plates were used as reference. Metal release from the coated surfaces was measured in water samples with inductively coupled plasma mass spectrometry (ICP-MS). Lethal toxicity assays were done on three marine species: the amphipod *Monocorophium insidiosum* and the mussel *Mytilus galloprovincialis* pediveliger larvae, as biofouling target species, and the copepod *Acartia tonsa* as non-target species. Besides, *A. tonsa* was used to test the toxicity of the individual active coating compounds.

FR coating lixiviate did not have an effect on the survival of any of the tested species, while BC coating lixiviate lead to mortality at relatively low concentrations for *A. tonsa*, but higher concentrations are needed for the other two tested species, suggesting that the response is species specific. In fact, *M. insidiosum* was the most tolerant species to BC coating, a finding that could have important implications for biosecurity management. Ongoing chemical analysis of obtained lixiviates will allow us to better understand the chemical agents potentially involved in the observed toxic responses and the behaviour of coating paints in different environmental scenarios. In conclusion, this work sheds light into biofouling control, providing insights into coating selection, performance and environmental risks.

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#### **1.09.A.T-05 The tire rubber derived-contaminant 6-PPD-quinone and increased temperatures: too much for the freshwater snail *Radix balthica***

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There is an increasing concern into the occurrence and fate of tire wear related compounds (TWCs) in the environment. One important class of TWCs are N, N'-disubstituted phenylenediamines, which are antioxidants added to tires to prevent the degradation of the rubber. They are highly reactive and this results in various transformation products. Among the globally used PPDs, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is one of the most frequently used tire antioxidant [1]. Recently, Tian et al. (2022) [1] identified a highly toxic quinone transformation product of 6PPD, 6PPD-quinone (6PPD-Q), as the toxicant responsible for acute mortality of the Pacific Northwest coho salmon (*Oncorhynchus kisutch*). These chemical stressors may interact with other global stressors affecting aquatic systems. For example, within the context of climate change, high temperatures and lower river discharges will cause water warming. Warming combined with chemical stressors may result in lower fitness in natural populations.

In order to test the toxicity of 6-PPD-Q in invertebrates in the context of climate change, we carried out a 10 days microcosm experiment with the freshwater gastropod *Radix balthica*. We tested the effects of 30 µg/L of 6-PPD-Q at two different temperatures, 15 and 20°C. As preliminary results, negative sublethal effects of the exposure to 6PPD-Q on growth and reproduction have been observed. Although the concentration tested (30 µg/L) is higher than the levels detected in aquatic systems, the quantitative data for this contaminant are still scarce, and in the context of climate change, we can expect to find high levels of TWCs in the future. In fact, we have seen that the effects of exposure to this antioxidant are accentuated by the effect of the increased temperatures. Thus, climate change may worsen the negative effects of the contaminant and compromise the survival of *R. balthica* populations in polluted aquatic systems.

#### **1.09.B Invertebrate Species in Ecotoxicology: Challenges and Opportunities in a Global Change Scene**

### 1.09.B.T-01 Enhanced Tolerance to Narcosis in Starved *Daphnia magna* Neonates

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*Daphnia magna* is a standard model in ecotoxicity testing. The test protocols and guidelines specify the assay conditions, including feeding, during exposure to ensure reproducible test outcomes. In contrast, there is no explicit standardisation for pre-exposure feeding despite the recognised significance of test organisms' energy reserves in coping with various stressors, including organic pollutants with narcosis as the main mode of action. This gap in standardisation can contribute to inconsistent test outcomes undermining reliable hazard assessments.

Here, we examined whether the pre-exposure feeding regime of neonates (<24 h) of *D. magna* can affect the test outcome in an acute dose-response experiment with chemicals inducing narcosis. The neonates were subjected to two alternative feeding regimes during the pre-exposure: starved (food-free media) and fed (algae present). Following this treatment, the daphnids were exposed for 72 h to a mixture of polycyclic aromatic hydrocarbons and then allowed to recover for 48 h in clean media with algal food. We measured survival and individual protein content in the daphnids at the end of each part of the experiment, i.e., pre-exposure, post-exposure and post-recovery. There were significant effects of the pre-exposure feeding manifested as (i) lower and less variable individual protein content, (ii) higher survivorship, and (iii) greater potential for recovery in the starved individuals. These findings imply that feeding during the first hours of daphnid life rapidly increases metabolically active body mass, with downstream effects on stress tolerance. Therefore, the pre-exposure feeding regimen plays a pivotal role in experimental design, especially when inducing test outcomes through narcosis, exerting a substantial influence on both the effect concentrations and their variability.

### 1.09.B.T-02 Transgenerational effects of the fluoroquinolone Flumequine in *D. magna*: An In Vivo Whole-Transcriptomic Investigation

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Flumequine (FLU), a long-standing antimicrobial widely used in aquaculture, lacks a comprehensive understanding of its toxicological effects on non-target species, especially long-term ones. This study delves into the potential transgenerational effects of FLU on *Daphnia magna*, a key crustacean model species in ecotoxicology. F0 generation was exposed for 21 days to two FLU concentrations (2.0 mg L<sup>-1</sup> and 0.2 mg L<sup>-1</sup>). Subsequent generations (F1 to F3) were kept for 21 days in pure medium. All the four generations (F0-F3) were subjected to phenotypic assessments, while the transcriptional profiles were investigated by means of RNA-sequencing in F0 and F3 generations. The results observed in the F3 generation were compared with those outlined in F0 and previously published. As to the FLU impact on phenotype, it decreased over generations. In contrast, in F3 generation, the transcriptional effects were exacerbated, suggesting a greater impact of FLU on progeny than on directly exposed organisms. Indeed, in F3 daphnids we observed 473 and 1187 differentially expressed genes (DEGs) following FLU 0.2 mg L<sup>-1</sup> and FLU 2.0 mg L<sup>-1</sup> exposure, respectively, while in F0 generations DEGs were 43 and 357 for low and high FLU dose, respectively. Functional analyses highlighted the upregulation of pathways related to cell duplication, nucleic acids binding, duplication and translation, coupled with the downregulation of pathways involved in the formation of the organism's nervous and structural systems.

These findings, indicating amplification of FLU transcriptional effects over generations upon reintroduction into a pure medium, suggest a potential adaptation to FLU contamination. It is worth noting that this potential adaptation was not appreciated at the phenotypic level, thus highlighting the importance of implementing molecular tools in risk assessment. Whether the observed differences in expression result from alterations at the DNA level (e.g., epigenetic factors) or a combination of other factors is still uncertain and worth of further investigations.

In conclusion, our research significantly contributes to understanding risks associated with the extensive use of fluoroquinolones in aquaculture and shows the potential transgenerational effects of environmental pollutants in aquatic systems.

### 1.09.B.T-03 An Open-Source Modular Workflow for Automated *Daphnia* Measurements of Sublethal Effects

*Philipp Kropf*<sup>1</sup>, *Matthias Schott*<sup>2</sup> and *Magdalena M. Mair*<sup>3</sup>, (1)University of Bayreuth, Germany, (2)Animal Ecology I, University of Bayreuth, Germany, (3)Statistical Ecotoxicology, University of Bayreuth, Germany

Large standardized datasets are becoming increasingly important for early environmental screening and the comparison of effects across different pollutants. While standardized procedures are usually available for simple acute mortality (e.g. OECD guidance 202) and chronic reproduction (e.g. OECD guidance 211) tests, test setups for other sub-lethal endpoints are often more diverse and not standardized. In addition, the testing of sub-lethal effects is usually time-consuming and subjected to potential observer bias, which hampers the implementation in high- or medium-throughput workflows. New *in silico*-based automation techniques that allow for standardized and fast measurements are often following very specific tasks that restrict their application to specific research questions, or lack open and freely usable code.

We developed a free open image analysis framework that is able to achieve both, standardized time-efficient measurements of *Daphnia*, and the option for modular extensions to adjust the workflow to more specific research questions. We use a Fast

Region-based Convolutional Network (Fast-RCNN) algorithm to detect *Daphnia* body parts (whole daphnid, head, eye, body, spina base, spina tip, heart, brood cavity) and measure body size (body length, body width, spina length) with human precision. The workflow is packed into a user-friendly function that allows for the analysis of a batch of images in a single line of code. At its core, the output contains visualizations of annotated images and a simple csv file with measurements. In addition to this basic user level, we demonstrate how the workflow can be extended flexibly to answer additional research questions and how it can be connected to other automatized measurement approaches. The program is provided as docker image and as source code on github.

#### **1.09.B.T-04 Investigating the fitness consequences of phenanthrene exposure in resurrected *D. magna* strains: a paleoecotoxicological study**

**Florian Gigl<sup>1</sup>**, **Muhammad Abdullahi Mr<sup>2</sup>**, **Marianne Barnard<sup>2</sup>**, **Henner Hollert<sup>3</sup>** and **Luisa Orsini<sup>2</sup>**, (1)Evolutionary Ecology & Environmental Toxicology, Goethe University Frankfurt, Germany, (2)School of Biosciences, University of Birmingham, United Kingdom, (3)Evolutionary ecology and environmental toxicology, Goethe University Frankfurt, Germany

Polycyclic aromatic hydrocarbons (PAHs) are a ubiquitous class of chemicals formed by the incomplete combustion of wood, crude oil, coal, and gasoline. Due to their lipophilic properties, PAHs spread ubiquitously and are persistent in the environment. Because of their biochemical properties, PAHs can accumulate in animal tissue with mutagenic, carcinogenic, and reprotoxic effects. Since the industrial revolution, PAHs have increased in the environment considerably, with concentrations ranging between 0.159 to 33090 µg/kg in lake sediments worldwide. To date, many studies have investigated the acute and chronic effects of PAHs on freshwater organisms. However, the long-term impact of these contaminants on fitness and ecological endpoints has been largely ignored. Moreover, it is unclear how prior exposure to other contaminants in the environment may alter the toxic response of organisms to these ubiquitous contaminants. Here, we study the impact of Phenanthrene (PHE), one of the most abundant PAHs in aquatic environments, on the sentinel and keystone species *Daphnia magna*. Capitalizing on the long dormancy of this species, we expose *D. magna* strains with different history of exposure to chemicals to PHE and measure its impact on fitness-linked life history traits. The studied temporal populations of *D. magna* were resurrected from a lake with well-paced and well-described history of chemical exposure. This allowed us to study fitness responses of both strains naïve to chemical stress and strains that have experienced historical exposure to chemical stress. Our study shows that both naïve and experienced populations to chemical stress suffer a fitness cost following exposure to PHE, showing lower fecundity, a delay in maturation and smaller size.

#### **1.09.P-Mo061 Toxicokinetic profiling of ZnO:Mn multicomponent nanomaterials in *Daphnia magna***

**Marianne Houin<sup>1</sup>**, **Maud Phillips<sup>1</sup>**, **Sébastien Cambier<sup>1</sup>**, **Johanna Ziebel<sup>1</sup>**, **Patricia M.A Farias<sup>2</sup>**, **Olavo D. F. Cardozo<sup>3</sup>**, **Andreas Stingl<sup>4</sup>**, **Arno C. Gutleb<sup>1</sup>** and **Kahina Mehennaoui, PhD<sup>1</sup>**, (1)Erin, Luxembourg Institute of Science and Technology (LIST), Luxembourg, (2)Postgraduate Program on Materials Science, PGMTR, CCEN, Federal University of Pernambuco, UFPE, Cidade Universitária, Brazil, (3)Phornano Brasil Ltda, Brazil, (4)Phornano Holding GmbH, Austria

Multicomponent nanoparticles (MCNMs) are emerging nanomaterials (NMs) developed by combining individual nanoparticles (NPs) to enhance their properties. Despite their prevalence, there is limited knowledge about the behaviour, fate, and potential effects of MCNMs on aquatic organisms. Zinc oxide (ZnO) is a widely used NPs, particularly in cosmetics and sunscreens. When coupled with manganese (Mn) NPs, ZnO is anticipated to exhibit increased stability and potentially reduced toxicity. To explore this hypothesis, *Daphnia magna* were exposed for 72h to ZnO NPs and to a MCNMs consisting of ZnO:Mn NMs. Toxicokinetic profiles of Zn and Mn were assessed after a 72h exposure period.

Organisms were first exposed for 24h to 300 µg.L<sup>-1</sup> of ZnO and ZnO:Mn before being transferred to a clean medium for 48h. Survival, Zn and Mn bioaccumulation and/or depuration, and behavioural responses were investigated. This approach provides comprehensive insights about the short term effects, uptake, and potential recovery patterns of *D. magna* exposed to ZnO and ZnO:Mn.

A rapid uptake of Zn was observed in organisms exposed to ZnO NPs and ZnO:Mn with 400 µg.g<sup>-1</sup>d.w. of Zn measured in organisms tissues after 1h of exposure and an uptake rate of 50.68 h<sup>-1</sup> and 68.52 h<sup>-1</sup> for ZnO NPs and ZnO:Mn respectively. Similarly, a rapid accumulation of Mn was observed after 1h of exposure of *D. magna* to ZnO:Mn with an uptake rate of 231.3 h<sup>-1</sup>. After 24h of exposure, organisms were transferred into clean medium. No Zn elimination was observed in both exposure condition, while a slow but significant elimination of Mn was observed in organisms exposed to ZnO:Mn. A significant effect on *D. magna* behaviour was observed. Mobility of exposed organisms was significantly lower compared to the control group after 48 and 72h of exposure, suggesting a delayed effects of ZnO and ZnO:Mn. Similarly, *D. magna* velocity was significantly lower in exposed conditions when organisms were in clean medium. The application of a light stimuli had significant effects only on non-exposed organisms highlighting the impact of ZnO and ZnO:Mn on *D. magna* phototaxis. The current study gives a better understanding of the exposure kinetics of ZnO and ZnO:Mn and their potential toxicity on *D. magna*, as they may have deleterious effects on population dynamics which may alter the functioning of aquatic ecosystems.

#### **1.09.P-Mo062 When *Daphnia* glow and tell us what's wrong: An Image-based screening method using Calcein AM in whole organisms for rapid assessment of chemicals**

**Amira Perez**, **Cedric Abele**, **Paula Pierozan**, **Andrey Höglund**, **Oskar Karlsson** and **Magnus Breitholtz**, Environmental Science, Stockholm University, Sweden

More than 86 million tons of chemicals, potentially hazardous to the environment, are produced annually in Europe. This



makes it nearly impossible to evaluate the adverse effects of each chemical in a realistic time frame. It is therefore fundamental to develop new tools capable of rapidly revealing adverse effects by quantifying relevant ecotoxicological endpoints.

New assessment methods (NAMs) such as image-based screening have started to evolve as an important predictive tool in ecotoxicology. Calcein AM is a lipophilic dye that permeates viable cells. Once in the cytoplasm it gets hydrolysed by the intracellular esterase into the fluorophore calcein, which emits a bright green signal. Cell viability as measured by intracellular esterase activity is a recognized parameter of cell health. Applying Calcein AM combined with automated image analysis in the model organism *Daphnia magna* is a novel approach that allows visualization of chemical effects in an intact organism. *D. magna* is a commonly used standard test species for ecotoxicological assessment of chemicals.

The aim of this project was to develop an image-based screening protocol for sublethal adverse effects in *D. magna* using Calcein AM. Esterase activity signal was analysed and quantified using Calcein AM after 24 hours exposure to three different model compounds known to have specific effects in *D. magna*: carbofuran and triphenyl phosphate interfering with the esterase activity and the insecticide methoxychlor, known to alter the viability and locomotor behaviour of *D. magna*. The signal intensity in each exposure was used to calculate EC<sub>50</sub>-values, which were compared to corresponding EC<sub>50</sub>-values obtained from the traditional OECD acute immobilization test.

Results showed a decreasing fluorescence intensity equal or similar to zero from Calcein AM with increasing concentration of Methoxychlor: 50-200 µg/L. These concentrations did not cause mortality. The EC<sub>50</sub> obtained by the Calcein AM signal intensity was 28.9 µg/L compared to 171 µg/L from the OECD immobilization test, which demonstrates a higher sensitivity of the image-based screening method.

This novel whole organism image-based screening NAM offers a rapid and sensitive tool to assess sublethal ecotoxicological effects of chemicals.

#### **1.09.P-Mo063 Impact of Antineoplastic Agents in the Survival and Cellular Homeostasis of *Daphnia magna***

Ana Carolina Matias<sup>1</sup>, Madalena Vieira<sup>1</sup>, Andreia Rodrigues<sup>2</sup>, Roberto Martins<sup>3</sup>, Susana Loureiro<sup>3</sup> and Maria D Pavlaki<sup>4</sup>, (1)University of Aveiro, Portugal, (2)CESAM & Department of Biology, University of Aveiro, Portugal, (3)CESAM-Centre for Environmental and Marine Studies and Department of Biology, University of Aveiro, Portugal, (4)University of Aveiro & Centre for Environmental and Marine Studies (CESAM), Portugal

Antineoplastic agents, which are vital components of cancer chemotherapy, are designed to inhibit tumor cell proliferation by interacting with DNA and modifying cellular growth factors. The release of these agents into municipal and hospital effluents has raised concerns regarding the potential risks to non-target aquatic organisms, particularly when they coexist in complex mixtures. This study aimed to investigate the effects of two distinct antineoplastic agents, doxorubicin (DOX) and oxaliplatin (OXA), on the acute effects of *Daphnia magna*, measuring immobilisation as an endpoint, and its sub-lethal antioxidant, biochemical, and neurotransmission responses, to evaluate the impact of their single and mixture toxic effects. Combined toxicities were predicted using two distinct models, Concentration Addition and Independent Action, along with possible deviations, such as synergism or antagonism, and effects dependent on “dose level” or those dependent on “dose ratio”. Acute effects of single exposure of the two antineoplastic agents to *D. magna* after 48 hours occurred at EC<sub>50</sub>=0.79 mg/L of DOX and at EC<sub>50</sub>=59.61 mg/L of OXA. Mixture exposure of DOX and OXA displayed a better fit for dose ratio deviation patterns for both models, showing synergism at higher OXA concentrations, whereas antagonism was mainly caused by increasing DOX concentrations. Biomarkers for neurotoxicity, oxidative stress, and cell membrane damage were evaluated in *D. magna* exposed to sublethal concentrations of DOX and OXA. Biomarkers included AChE, CAT, GPX, GR, GST, ETS, CYP c reductase, CYP3A4 activity, and LPO. Alterations in LPO were observed for DOX exposure, while CAT, GPx, GR, GST, and ETS showed changes with OXA exposure. Mixture exposure of DOX and OXA resulted in alterations in ETS and AChE. The ecotoxicological data presented in this study not only highlights the potential environmental risks associated with anticancer drugs, particularly concerning their synergistic effects but also emphasizes the importance of integrating statistical models to assess the environmental impact of these compounds, as their behaviour will be different from the impact of single stressors.

#### **1.09.P Invertebrate Species in Ecotoxicology: Challenges and Opportunities in a Global Change Scene**

##### **1.09.P-Mo061 Toxicokinetic profiling of ZnO:Mn multicomponent nanomaterials in *Daphnia magna***

Marianne Houin<sup>1</sup>, Maud Phillips<sup>1</sup>, Sébastien Cambier<sup>1</sup>, Johanna Ziebel<sup>1</sup>, Patricia M.A Farias<sup>2</sup>, Olavo D. F. Cardozo<sup>3</sup>, Andreas Stingl<sup>4</sup>, Arno C. Gutleb<sup>1</sup> and Kahina Mehennaoui, PhD<sup>1</sup>, (1)Erin, Luxembourg Institute of Science and Technology (LIST), Luxembourg, (2)Postgraduate Program on Materials Science, PGMTR, CCEN, Federal University of Pernambuco, UFPE, Cidade Universitária, Brazil, (3)Phornano Brasil Ltda, Brazil, (4)Phornano Holding GmbH, Austria

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This novel whole organism image-based screening NAM offers a rapid and sensitive tool to assess sublethal ecotoxicological effects of chemicals.

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*Ana Carolina Matias<sup>1</sup>, Madalena Vieira<sup>1</sup>, Andreia Rodrigues<sup>2</sup>, Roberto Martins<sup>3</sup>, Susana Loureiro<sup>3</sup> and Maria D Pavlaki<sup>4</sup>, (1)University of Aveiro, Portugal, (2)CESAM & Department of Biology, University of Aveiro, Portugal, (3)CESAM-Centre for Environmental and Marine Studies and Department of Biology, University of Aveiro, Portugal, (4)University of Aveiro & Centre for Environmental and Marine Studies (CESAM), Portugal*

Antineoplastic agents, which are vital components of cancer chemotherapy, are designed to inhibit tumor cell proliferation by interacting with DNA and modifying cellular growth factors. The release of these agents into municipal and hospital effluents has raised concerns regarding the potential risks to non-target aquatic organisms, particularly when they coexist in complex mixtures. This study aimed to investigate the effects of two distinct antineoplastic agents, doxorubicin (DOX) and oxaliplatin (OXA), on the acute effects of *Daphnia magna*, measuring immobilisation as an endpoint, and its sub-lethal antioxidant, biochemical, and neurotransmission responses, to evaluate the impact of their single and mixture toxic effects. Combined toxicities were predicted using two distinct models, Concentration Addition and Independent Action, along with possible deviations, such as synergism or antagonism, and effects dependent on "dose level" or those dependent on "dose ratio". Acute effects of single exposure of the two antineoplastic agents to *D. magna* after 48 hours occurred at  $\text{EC}_{50}=0.79$   $\text{mg}/\text{L}$  of DOX and at  $\text{EC}_{50}=59.61$   $\text{mg}/\text{L}$  of OXA. Mixture exposure of DOX and OXA displayed a better fit for dose ratio deviation patterns for

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#### **1.09.P-Mo064 The Sub-lethal effects of Water Accommodated Fraction from chemically dispersed Marine Gas Oil on the North Atlantic copepod *Calanus finmarchicus***

**Tamer Amr Hafez<sup>1</sup>, Maren Ortiz-Zarragoitia<sup>2</sup>, Dag Altin<sup>3</sup>, Zdenka Bartosova<sup>4</sup>, Bjørn Munro Jenssen<sup>5</sup> and Tomasz Maciej Ciesielski<sup>4</sup>,** (1)Zoology and Animal Cell Biology, CBET+, PiE, University of the Basque Country UPV/EHU, Spain, (2)Research Centre for Experimental Marine Biology and Biotechnology (Plentzia Marine Station; PiE-UPV/EHU), University of the Basque Country (UPV/EHU), Spain, (3)Research Infrastructure SeaLab, Norwegian University of Science & Technology (NTNU), Norway, (4)Norwegian University of Science & Technology (NTNU), Norway, (5)Department of Biology, Norwegian University of Science & Technology (NTNU), Norway

Due to the maritime regulations to reduce sulfur emissions, there has been a shift in use of fuels with low sulfur content. As a result, the use of the low sulfur Marine Gas oil (MGO) has increased in the shipping industry. Although several studies have investigated the toxicity of MGO through Water Accommodated Fractions (MGO WAF), few investigated how chemically enhanced MGO WAF (MGO CEWAF), by the application the dispersant (FinaSol OSR52,10%), modifies the toxicity to marine organisms in cold environments. The copepod *Calanus finmarchicus* is an important component of the zooplankton communities of the North Atlantic and is a valuable food source for commercially important fish. In addition, *C. finmarchicus* has been extensively studied concerning hydrocarbon contamination, making it a model organism to investigate the effects of MGO CEWAF. Hence, the objective of this study was to investigate sublethal effects of MGO CEWAF on *C. finmarchicus*. Adult *C. finmarchicus* were exposed to a sublethal concentration of MGO CEWAF corresponding to 50% of the calculated LC<sub>50</sub>-value after 96 hours of exposure (4.22% MGO CEWAF in seawater) for 24, 48, 72 and 96 hours. Sublethal responses were assessed by quantifying the transcription levels of several biomarker genes, activities of antioxidant enzymes, lipid peroxidation levels, and accumulation of the carotenoid astaxanthin. Gene transcription analysis showed significant downregulation of the biotransformation gene *cyp330A1* in all the exposed groups compared to control groups. Furthermore, changes in the gene transcription levels of elongase, fatty acid binding protein and ferritin suggested that exposure to MGO CEWAF affected lipid metabolism and consequently affect the copepods' ability to store energy and successfully complete diapause, an important phase in the copepod's life cycle. In addition, the significant reduction in activities of antioxidant enzymes, such as catalase, superoxide dismutase and total glutathione, suggests that exposure to MGO CEWAF induced oxidative stress. The reduced capabilities to oxidative stress regulation and changes in lipid metabolism will be further investigated through levels of lipid peroxidation and the amount of accumulated astaxanthin.

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#### **1.09.P-Mo065 Developing a Protocol for Measuring Viability and DNA Damage in Sperm Cells of Marine Amphipods *Marina Tenório Botelho<sup>1</sup> and Gisela de Aragão Umbuzeiro<sup>2</sup>,*** (1)School of Technology – State University of Campinas, Brazil, (2)Faculdade de Tecnologia, Universidade Estadual de Campinas (UNICAMP), Brazil

Adverse effects in germ cells are crucial endpoints to enable a comprehensive chemical hazard evaluation. Reduced sperm quality has been associated with negative effects at population levels in aquatic organisms. There is a lack of protocols for viability and DNA damage evaluation in sperm cells especially for marine invertebrates. *Parhyale hawaiiensis*, a tropical marine amphipod, has been recognized as a promising model in ecotoxicology. So, this work aimed to optimize protocols to evaluate sperm viability and DNA damage (comet assay) using *P. hawaiiensis* for *in vivo* experiments. Sperm viability and comet assay evaluations were performed after exposure of adults (8 months) to selected mutagenic compounds for 24h and 96h. Organisms were anesthetized and dissected to remove the testes, which were then dilacerated in artificial seawater to obtain sperm cell suspensions. To evaluate viability, sperm cells were stained with SYBR-14 and propidium iodide and counted under a fluorescent microscope after 24h exposure. To evaluate sperm cell DNA damage, standard alkaline comet assay protocol (EtBr stain) was used with organisms exposed for 24 and 96h. For viability, we evaluated the mutagen ethylmethane sulfonate (EMS) from 0.1 to 2 mM and dimethyl sulfoxide (DMSO) from 0.01 to 1%. We wanted to evaluate the maximum concentration that could be used without decreasing viability, since most organic toxicants use DMSO as solvent. No reduction in viability was observed for EMS and DMSO. To verify the best time of lysis and unwinding in the comet assay, we direct exposed sperm cells to EMS and UV radiation and verified that the used protocol provided satisfactory responses and the best conditions were 18h of lysis and 15 min of unwinding. When we compared the DNA damage responses of sperm cells and hemocytes for both mutagens at the same exposure times, sperm cells were more sensitive. Then, we exposed 12 males per concentration of EMS (0.1 to 2mM) for 24 and 96h. A clear concentration response was observed after 24h, but for 96h all concentrations provided high percentage of damage (>70%). Both viability and DNA damage protocols were successfully developed and will be now applied to evaluate mutagens that require metabolic activation, such as PAHs, azo and

anthraquinones dyes. It seems that 24h exposure time is sufficient to detect DNA damage (comet assay) at least for direct mutagens as EMS. For other compounds different exposure times may be needed.

#### **1.09.P-Mo066 Framework for Immunotoxicological Studies using the Marine Amphipod *Parhyale hawaiiensis*: Hemocytes Characterization**

*Amanda dos Santos*<sup>1,2</sup>, *Marina Tenório Botelho*<sup>3</sup>, *Willian Reina Joviano*<sup>4</sup>, *Vicente Gomes*<sup>4</sup>, *Jose Roberto Silva*<sup>4</sup> and *Gisela de Aragão Umbuzeiro*<sup>5</sup>, (1)Center for Energy and Petroleum Studies - University of Campinas, Brazil, (2)School of Pharmaceutical Sciences - São Paulo State University, Brazil, (3)School of Technology – State University of Campinas, Brazil, (4)University of São Paulo (USP), Brazil, (5)Faculdade de Tecnologia, Universidade Estadual de Campinas (UNICAMP), Brazil

Hemocytes are blood cells present in the hemolymph of amphipods and other crustaceans and are very important to the functioning of the immune systems. Given the importance of the amphipods as test organisms in ecotoxicological studies and the possibility of collecting hemolymph from them, several studies have used this matrix to assess DNA damage after different types of exposures. Nevertheless, the knowledge about the types of cells found in the hemolymph or their relative quantities from these investigations is limited. Hemocytes of the marine circumtropical amphipod *Parhyale hawaiiensis* have been used for the evaluation of DNA damage and micronuclei, but they have not been characterized in the scientific literature. Thus, this investigation aimed to characterize *P. hawaiiensis* hemolymph cells and examine their phagocytotic activity. Basic dyes, such as Rosenfeld and Sudan Black, were selected to differentiate the cell types and the presence of lipids. Three parameters were established: total hemocyte counts (THCs), the proportion of hemocyte types, and their size. To verify phagocytosis activity, hemolymph was exposed to *Escherichia coli*. We observed three distinct cell types in *P. hawaiiensis* and all of them contained lipids. Granulocytes had an oval shape and size of  $13.4 \times 7.6 \mu\text{m}$ , semi-granulocytes also had an oval shape and a similar size ( $14.1 \times 7.2 \mu\text{m}$ ), and hyalinocytes had a round shape and a smaller size ( $9.6 \times 7.2 \mu\text{m}$ ). The proportions of the three cell types varied between males (64.8%, 31.1%, and 4.2%) and females (70.1%, 28.2%, and 1.7%). Males had significantly higher THCs than females (males:  $9007 \pm 3800$  cells per individual; females:  $4695 \pm 1892$  cells per individual). It was observed that hemocytes were able to phagocytose *E. coli*, indicating that assays based on phagocytosis could also be explored as an immunotoxicity endpoint. These results advance in the understanding of *P. hawaiiensis* hemocytes and contribute to the framework for hemocyte-based immune responses as an endpoint in ecotoxicology.

#### **1.09.P-Mo067 The toxic effect of plastic leachate on marine periwinkle *Littorina littorea***

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Plastic pollution poses a substantial threat to marine coastal ecosystems. Besides the physical effect of plastic debris and particles, plastic-associated chemical additives are continuously released from the polymeric matrix and can cause toxicity for marine benthic organisms. This release is enhanced by high coastal hydrodynamics and solar radiation. However, the way these complex mixtures of leached plastic-associated chemicals affect key littoral benthic species is still not fully understood. To investigate this, we exposed a common periwinkle, *Littorina littorea*, for three days to a dilution series of leachates prepared from UV-treated transparent polyethylene foil. The movements of snails were monitored by measuring the time of withdrawal from the shell, the time when tentacles appeared, and the time needed for the snails to rotate from the upward position. Additionally, the leachates were characterized using a set of complex analytical methods using liquid chromatography (LC) coupled to (high-resolution) mass spectrometry ((HR)MS). The results showed that the leachates suppressed the moving activities of snails in a dose-dependent manner. Metabolomic analysis using <sup>1</sup>H-Nuclear Magnetic Resonance (NMR) spectroscopy revealed that this suppression goes along with an increase in the concentration of biomarkers of anaerobic metabolism together with glucose depletion in pooled snail's tissues. This indicates a disturbed energy homeostasis induced by leachate exposure. The activity of acetylcholinesterase, a biomarker related to the nervous system, and levels of choline and serotonin changed after the exposure, indicating that the used leachate might have a neurotoxic effect on snails. This could explain the suppression of moving activity. The interconnection of the obtained results with the chemical composition of the used leachate is discussed in this intradisciplinary study.

#### **1.09.P-Mo068 BPE exposure of the snail *Lymnaea stagnalis*: investigating effects in embryos, adults and after parental exposure**

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Bisphenols, such as Bisphenol A (BPA), have been used for decades in plastics and resins. After BPA has been considered toxic to reproduction and has been listed on the EU Candidate List of Substances of Very High Concern, BPA-alternatives, such as Bisphenol E (BPE), have been developed. The adverse effects of these alternatives are investigated within the European Partnership for the Assessment of Risks from Chemicals (PARC). The OECD published a *L. stagnalis* reproduction test guideline (OECD TG 243), but tests on embryos are yet to be developed. We investigated the effect of BPE, on both *L. stagnalis* embryos and adults. Embryos were exposed 21 days to 0.1, 0.5, 1, 2, 5, and 10 mg/L of BPE. For the reproduction

test, adult snails were exposed in semi static conditions to five BPE concentrations, from 0.1526 to 1.6 mg/L, with a 3.2 factor. The BPE concentration was checked regularly in the exposure water using a UHPLC-3Q-MS/MS. We found that embryos exposed to 5 mg/L grew 21.5% less than the controls, and had significantly decreased heartbeat rate at day 6, but no death occurred. Embryos exposed to 10 mg/L grew 36.1% less than the controls. At this concentration, embryos didn't have heartbeat at day 6, and only 7% of the embryos had hatched after 21 days. However, in spite of severe malformations, no death occurred due to the exposure at 10 mg/L. For adults exposure, a range finding prior to the test showed a 100% mortality of the snails at 10 mg/L. The TG 243 experiment is ongoing. The chemical analysis results show a high instability of the BPE concentration, with down to only 20% of the nominal concentration left after 30h. During the reproduction test, eggs from exposed parents are collected in order to perform embryo tests, and evaluate the potential parental transfer of BPE toxicity. In conclusion, both *L. stagnalis* embryos and adults are sensitive to BPE. We also showed the importance of chemical analysis in exposure test to allow the comparison of results between laboratories and species. Results from embryo exposure tests are promising and will help evaluating the relevance of developing invertebrates embryo-based toxicity tests.

#### **1.09.P-Mo069 Effects of Culturing Conditions on Metal Toxicity to *Lymnaea stagnalis* - Focus on Diet**

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*Lymnaea stagnalis*, a prevalent freshwater snail, plays a crucial role in aquatic ecosystems and is well-recognized for its sensitivity to metals, particularly copper (Cu). As a key model organism for assessing metal exposure risks in freshwater environments, understanding the factors influencing its sensitivity is important. This study consists of two sequential experiments to evaluate the impact of experimental conditions (diet quality) on the sensitivity of *L. stagnalis* to Cu. In the initial experiment, newly-hatched snails were exposed to a range of Cu concentrations (1, 2, 5, 10, 20, 50, and 100 µg/L) over 37 days to determine the EC50. The second experiment explored the influence of diet quality on snail sensitivity to Cu. Four distinct diets, including romaine lettuce, sweet potatoes, fish flakes, and periphyton, were tested under Cu-free and Cu-contaminated conditions at the selected EC50. Results from the first experiment indicated significant effects of Cu exposure on growth rate and mortality. A concentration of 15 µg/L was selected for the second experiment based on the EC50. Interestingly, this concentration did not impact growth rate. However, diet quality was found to be a critical factor influencing growth. Indeed, growth rate responded to diet quality depending on development stage. This study presents a contribution to the improvement of methodologies and enhances the reliability of toxicity assessments in *L. stagnalis*, thereby informing more accurate risk assessments in ecotoxicological studies.

#### **1.09.P-Mo070 How can we Better Understand Ecosystem Scale Effects of Neuroactive Chemicals?**

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Whilst we know neuroactive chemicals are pervasive in the environment, we know little of their impact within freshwater ecosystems. Existing toxicity tests are principally undertaken in controlled laboratory conditions on a small number of species and focus on the individual-level effect of chemicals. As a result, our understanding of sublethal and indirect effects, of which there are potentially many (e.g., reduced feeding activity and subdued predator responses), is limited. A major challenge, therefore, is to understand the effects of neuroactive chemicals at community and network scales within freshwater ecosystems. A multi-method approach will be used to understand the risk posed by neuroactive chemicals to freshwaters. Firstly, we will complete a systematic review into the fate and effects of neuroactive chemicals. Secondly, we will assess the concentrations of neuroactive chemicals in local river catchments across South Wales, UK. Thirdly, we will interrogate mammal, fish and invertebrate genomic databases to identify orthologues for receptors that different neuroactive chemicals interact with and determine the degree to which certain chemicals are likely to have impacts across freshwater ecosystems. By combining the findings of all three analyses we will be able to identify the most prevalent and potentially toxic chemicals at both the global and local scale.

Using the information from the above in silico and field assessments we will employ a combination of aquatic micro- and meso-cosm systems, seeking to address two main questions: (1) how do neuroactive chemicals alter ecological interactions (e.g., predation and competition) and the complex networks these interactions form; and (2) do changes to ecological networks result in alterations in ecosystem functioning? These experiments, assessing the impact of neuroactive chemicals as individual compounds and in mixtures within multi-species systems, will further our understanding of their effects on the complex interactions across trophic levels. Ultimately, by combining both empirical and in silico approaches, we aim to generate much needed policy-relevant and environmentally realistic data to highlight the importance of incorporating behavioural end points with standard ecotoxicological tests to inform chemical risk assessment, regulation and wastewater management.

#### **1.09.P-Mo071 Assessing the Effects of Chemicals on UK-Relevant Freshwater Invertebrates**

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Freshwater macroinvertebrates are highly vulnerable to anthropogenic stressors such as chemical pollution, with one-third of freshwater insects under threat of extinction. However, macroinvertebrate species vary greatly in their sensitivity to different

chemicals, making it challenging to predict the impact of a given chemical on diverse freshwater invertebrate assemblages. Some of this interspecies variability is linked to a chemical's toxic mode of action (TMoA), wherein general acting chemicals (e.g., metals) exhibit smaller variations compared to specifically acting ones (e.g., insecticides). This interspecies variation in chemical sensitivity could result in variable assemblage sensitivity and associated risks from chemical exposure, a factor currently overlooked in existing assessments of chemical impacts. Given that it is not feasible to undertake toxicity tests for every species-chemical combination, our study introduces a novel strategy for obtaining effects data on high-priority chemicals for UK-relevant invertebrates, both laboratory cultured and field collected organisms. Our underlying hypothesis is that within a TMoA, the rank order of sensitivity of different species to chemicals remains constant, and the relative potency of chemicals across a TMoA is consistent. To test this hypothesis, we focused on six distinct chemical classes (metals, triazoles, polyaromatic hydrocarbons, synthetic pyrethroids, strobilurins), each characterized by a unique TMoA. We tested 3-4 chemicals from each class on an anchor species, *Daphnia magna* and tested 2-3 chemicals from each class on 10 distinct macroinvertebrate organisms. Macroinvertebrates were selected to represent the main taxonomic groups (e.g., arthropods, annelids, molluscs) and functional traits (e.g., detritivores, filter-feeders, grazers, predators) found within freshwaters assemblages. For each of these organisms, we conducted acute immobilization tests and investigated whether the rank order of sensitivity remains consistent within a chemical class. If our hypothesis is correct – that groups of similar species show consistent responses to particular chemical classes, this will facilitate the extrapolation of effects across species and chemicals. This will ultimately provide a more holistic and ecologically relevant perspective on the impact of chemicals on UK freshwater assemblages.

#### **1.09.P-Mo072 Implications of WWTP Upgrade on aquatic organisms: Gammarids and NGS**

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Wastewater treatments plants (WWTP) are known point sources for micropollutants (MP) in surface water systems. To reduce the introduction of MP in Switzerland, WWTPs are currently upgraded with further treatment steps. To demonstrate the impact of this measures on the receiving streams, which is a huge financial investment for the involved partners, currently the removal efficiency of 12 tracer MP is recommended. This practice is not satisfying since it's not representing the impact of the water quality improvement for water organisms directly. The goal of this project was to demonstrate if increase of water quality can be represented by using actively monitored gammarids and next generation sequencing (NGS).

For several years adult male gammarids from the respective nearby ecosystem were selected and introduced in an active in situ monitoring up and downstream of WWTP outlets before and after an upgrade of a WWTP (monitored sites n=4, 1-3y). The gammarids were exposed for 1 month including food. After exposure the gammarids were counted, the RNA of the whole organisms were isolated and used for Next Generation Sequencing (Illumina NextSeq 550). A workflow based on the Galaxy platform (<https://usegalaxy.org/>) for the data evaluation was established including a transcriptome assembly. The project is currently still running, but initial results after the (partial) upgrade of the first two WWTP showed, that the number of significantly regulated transcripts between upstream and down streams are reacting towards the change in water quality. For example, after the upgrade with a PAK-Treatment, the number of significantly regulated transcripts between up- and downstream was 3-times lower. The reduction pattern could be replicated in the subsequent year. Surprisingly, despite large biological variation, the number of significantly altered transcripts was comparable between up and down before and after removal at one site over 2 years.

At another WWTP a new biological treatment system has been activated, even with a still pending MP upgrade, a reduction of significantly regulated transcripts between up- and downstream could be demonstrated. For further analysis more WWTP upgrades are needed, but current results show that this "Non target" gene approach is showing a good responsiveness and could be an interesting indicator of water organisms health status, especially considering the high biological variance among populations and years

#### **1.09.P-Mo073 Effects of Biodegradable Microplastics on the crustacean isopod *Idotea balthica basteri* Pallas, 1772**

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Plastic pollution is a remarkable environmental issue, being plastic widespread and characterized by long lifetime. Serious environmental problems are caused by the improper management of plastic end of life. In fact, plastic litter is currently detected in any environment. Biodegradable polymers are promising materials if correctly applied and managed at the end of life, to minimize environmental problems. However, poor data on BPs fate and toxicity on marine organisms limit their applicability. In the present work we tested the effects of biodegradable polymers (polybutylene succinate, PBS; polybutylene succinate-co-butylene adipate, PBSA; polycaprolactone, PCL; poly (3-hydroxybutyrate), PHB; polylactic acid, PLA) widely used in various anthropic activities. These polymers were fed for 35 days to adult individuals of the isopod *Idotea balthica basteri*, by adding fragments to formulated feeds at two concentrations, i.e., 0.84 and 8.4 g/kg feed. Our results showed that the polymers affected the survival rates of the isopods as well as the expression levels of eighteen genes (tested by Real Time

qPCR) involved in stress response and detoxification process. Our findings demonstrated that *I. balthica basteri* is a convenient model organism to study the response to environmental pollution and to emerging contaminants in the aquatic environment, and highlight the need for the correct use of biodegradable plastic polymers.

#### **1.09.P-Mo074 Salinity fluctuations impact behavior of copepods: in situ monitoring using the multispecies freshwater biomonitor**

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Many organisms living in estuarine environments have evolved abilities to cope with changing salinities. The cyclopoid copepod *Apocyclops royi* are normally living in estuarine and coastal pond environments in e.g. Taiwan. Hence, they are during the monsoon season exposed to heavy rain falls and following decrease in salinity. At other times of the year, they experience longer heat periods and subsequent evaporating water, thus increasing salinities. This study examined the impact of salinity change on the activity and behavioral response of *A. royi*. More specifically we asked 1) how well does *A. royi* cope with extreme salinity changes (0 to 32 PSU), and 2) how long will it take to recover (from being inactive to be active again). Animal movements in the water generate specific frequencies, and the Multispecies Freshwater Biomonitor (MFB, Limco International) can estimate the percentage of time producing each frequency (from 0.5 to 8.5 Hz) by means of a stepwise discrete Fourier transformation. The chambers contain biosensors, which are placed on the inside wall. The biosensors come in pairs and there are two pairs in each chamber. One pair is sending out an alternating current through the water, which is then received by the other pair of sensors. If the chambers contain an animal, the movement of the animal will be detected by the change in amplitude of the signal sent by the first pair of sensors. MFB can be used in both marine- (0.1V) and freshwater (1V). *A. royi* was reared in a dark climate room in seawater (32 PSU) at 25°C and fed with the microalgae, *Rhodomonas salina* twice a week. Female *A. royi* with egg sacks was selected and placed individually in the experimental MFB chambers after addition of either DI- (0 PSU) or marine (32 PSU) water (n=8 chambers per salinity). Activity was recorded using the MFB: 1 V for 0 PSU and 0.1 V for 32 PSU. The MFB has 16 channels (8 for each salinity) and is based on the quadruple impedance conversion technique to record live organism behavior. We found that *A. royi* decrease activity significant and is almost inactive initially when transferred from 32 to 0 PSU. Further, they appear to regain activity within hours suggesting that they will be able to handle extreme salinity stress. However, it is still to be examined how *A. royi* react to the combined stress of salinity change and contaminant exposure.

#### **1.09.P-Mo075 Flow Cytometry as a Tool to Assess the Responses of *Mytilus edulis* Digestive Cells to Contaminants Present in the Arctic**

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Primary cell cultures from aquatic organisms are being increasingly developed for use in toxicity testing and mechanistic studies in response to xenobiotics. Whereas fish cells are commonly used, less is known about primary cells derived from aquatic invertebrates. Digestive glands play a key role in the metabolism and biotransformation of xenobiotics in mussels *Mytilus sp.*, making digestive cells relevant *in vitro* models for studying pathways and toxic mechanisms of xenobiotics. Flow cytometry (FCM) can be used as a tool to evaluate the toxicological effects of contaminants in individual cells close to an *in vivo* state. However, its potential use in integrated effects assessment and next generation risk assessment has not yet been fully explored. So, the aim of this study was to investigate the sensitivity of FCM to quickly screen the effects of model chemicals towards digestive cells isolated from *Mytilus edulis*. In a first assessment, the morphological, physiological and structural characteristics of digestive cells were determined using light microscopy, fluorescence microscopy and FCM. Several cell types were identified visually, comprised mostly of larger cells filled with lysosomes and smaller cells containing none to a few lysosomes or lipids. The presence of two main cell populations with different sizes and complexity was identified by FCM. Secondly, the integrity, viability and biochemical functions of isolated digestive cells were evaluated up to 24 hours post-isolation using FCM, for which mortality, cell viability, metabolic activity and neutral lipids and lysosomes presence were determined. Results obtained showed that digestive cells maintained their cellular integrity and biochemical functions over time, even though a small decrease in viability was seen at 24h post-isolation. Exposure experiments are currently ongoing, where digestive cells will be exposed to model chemicals, e.g., copper, pyrene, and the following endpoints determined by FCM: mortality, cell viability, metabolic activity, neutral lipids and lysosomes presence, ROS formation, membrane potential, lipid peroxidation and DNA content. Overall, the use of FCM seems to be a fast, accurate and reproducible cost-effective method to quickly assess a wide array of cellular functions in mussel digestive cells that can be integrated into assessment of contaminants present in the Arctic. This work was supported by the EXPECT Project (#315969) funded by the Research Council of Norway.

#### **1.09.P-Mo076 Can primary producers act as vector of nanoplastics bioaccumulation on small filter feeders?**

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Bioaccumulation is a key component for the assessment of nanoplastics risk to the environment. Within aquatic ecosystems, filter-feeding organisms are particularly relevant to sentinel for plastic contamination due to their highly specialized filtering

structures. Although varying greatly among different zooplanktonic species on their biological filter-mesh size and sensory adaptability, the range limits seems to be set by the structure and function of the filtering apparatus used for trapping particles. It is therefore interesting to ask: does the biological filter mesh-size of small filter feeders matters when bio-concentrating nano-size plastic particles?

Within an environmental context, small filter feeders tend to be foodborne-exposed to the contaminants. It is therefore highly relevant to discuss the ecological role of phytoplanktonic species to try to understand if these can contribute as vectors of bioaccumulation, since as primary producers it would not be firstly anticipated that these microorganisms occupying the first trophic level of every trophic chains would have an “intent” to uptake nanoplastics.

In this work, relevant planktonic species were directly collected from Lake Maggiore (Italy) and exposed to three gold-doped nanoplastics for 24 h, considering nonstandard experimental designs. A non-axenic microalgae culture containing a mixture of species was used as foodvector for the nanoplastics exposure on freshwater cladocerans and copepods. Contact tests of freshwater microalgae to the nanoplastics were conducted at the exponential growth phase for 24 h and several relevant biomarkers were assessed. Metal-doped nanoplastics bioaccumulation on these microorganisms was assessed by ICP-MS analysis. Taken together, the obtained results pinpoint that zooplankton when fed with nanoplastics only (no microalgae) tend to accumulate significant levels on their bodies, however, when co-exposed with microalgae a decrease in the accumulated content was recorded. As microalgae directly exposed to the nanoplastics demonstrated high levels of metal content, it is reasonable to anticipate that the lower levels uptaken by the zooplankton was due to competition among species. Moreover, interesting differences on bioaccumulation levels among the two zooplanktonic species were observed, which seems to reflect their different biological filtering mesh-sizes. At last, differences on nanoplastics bioaccumulation were also demonstrated between different particles.

#### **1.09.P-Mo077 Sublethal Effects On Planktonic Biota Of New Phosphorus Adsorbents Used For Lake Restoration**

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Currently, it is well-known that eutrophication, characterized by an increase in the primary production of aquatic ecosystems, represents one of the major challenges for the quality of water resources worldwide. This also leads to a loss of biodiversity and disrupts aquatic ecosystems, emphasizing the importance of restoring these environments to aid in biodiversity recovery. Phosphorus is typically the primary limiting nutrient for primary production in most inland aquatic ecosystems. Therefore, reducing its concentration in water stands as the most crucial strategy to control eutrophication. In this context, short-term standardized laboratory tests were conducted to assess the sublethal effects on aquatic biota of two novel phosphorus adsorbents: carbonyl iron magnetic particles (HQ) and lanthanum-modified bentonite (Phoslock®). A series of toxicological tests were run by using species from two different trophic levels: primary producers such as *Desmodesmus* sp. (measuring algal growth rate inhibition) and primary consumers such as *Daphnia magna* (evaluating filtration and somatic growth rate). At this point, it is important to consider that adsorbent doses used in these experiments were much higher than doses expected to be used in a real scenario based on mobile sedimentary phosphorus and maximum adsorption capacity for phosphorus for each adsorbent. Therefore, if no effects on organisms are found at these high doses, no effects are likely to be expected at lower concentrations. Our results initially indicated that, in algal growth rate experiments, no inhibition was observed for HQ or Phoslock® despite the significant reduction (albeit still above the metabolic threshold) in phosphate concentrations upon adsorbent addition. *Daphnia* filtration rate experiments revealed that neither HQ nor Phoslock® had negative effects through indirect contact. However, a significant reduction in the somatic growth rate of *Daphnia* was observed when both adsorbents were directly added in all concentrations, particularly with Phoslock®, resulting in a more pronounced reduction at highest concentrations (1 and 2 g L<sup>-1</sup>). Nonetheless, indirect contact with both adsorbents did not yield any significant effects on the somatic growth of *Daphnia*. Overall, the addition of these adsorbents in a real lake-wide application is expected to cause minor sublethal effects in *Desmodesmus* and *Daphnia*.

#### **1.09.P-Mo078 Indirect effects in mudsnails through dietary uptake?**

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Aquatic ecosystems are characterized by multiple connections within and among trophic levels. Chemical stress can disrupt these interactions and lead to shifts in structure and function. Pesticides or pharmaceuticals can enter surface waters via different pathways such as agricultural runoff or sewage treatment plants. Direct effects of these chemicals towards aquatic primary producers such as biofilms have been widely studied. Biofilms not only contribute to primary production, but also represent an important food source for grazing animals and host a large biodiversity including bacteria and algae. Studies of potential indirect effects towards higher trophic levels (primary consumers) are scarcer. We therefore hypothesized that species turnover in the biofilm community composition could in turn indirectly affect grazers feeding on biofilm. We studied river biofilms as food source for the grazing New Zealand Mudsnail *Potamopyrgus antipodarum* after exposure to chemical stress. *P. antipodarum* is a globally widespread invasive grazing gastropod, that lives in freshwater and consumes food via scraping off organic coatings. The mudsnail has been established and widely used in the OECD 242 reproduction test since 2016. In



terms of chemicals, we chose an herbicide to directly address the algal species and an antibiotic to address the bacterial community within the biofilm.

We collected biofilm from a small river in a natural reserve in Germany as an inoculum to colonise ceramic tiles in stream microcosms. After four weeks of colonisation, biofilms were chronically (14 days) exposed to 1) an unexposed control, 2) 10 µg/L of propyzamide, 3) 10 µg/L of ciprofloxacin and 4) to the mixture of both. In subsequent feeding assays, grazers were fed *ad libitum* with those biofilms for 21 days. Grazers were analysed regarding their physiological condition (fatty acid composition) and feeding activity, while the biofilm was analysed in terms of biomass, chlorophyll content, and fatty acid composition. The data is currently being analysed supporting a coherent interpretation of these indirect effects. Based on the above hypotheses, we expect chemically induced changes in the biomass and nutritional quality (less essential macronutrients such as highly unsaturated fatty acids) of the biofilm. Consequently, we also expect to see indirect changes in the snails based on an altered feeding rate and a similarly altered fatty acid profile compared to the control approaches.

#### **1.09.P-Mo079 The Impact of Environmental Factors on the Growth and Development of a Laboratory Culture of *Cloeon dipterum***

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In the European Union (EU), non-standard test species are used in the refinement of aquatic risk assessments of plant protection products (PPPs). Representatives of EPT taxa (Ephemeroptera, Plecoptera and Trichoptera) are of particular interest as they are classed as sensitive and vulnerable to PPPs, however, they are known to be difficult to work with in a laboratory setting and the seasonality of their life cycle restricts the frequency and scale of ecotoxicity testing.

The mayfly *Cloeon dipterum* is a highly abundant species across the UK and Europe, residing in ponds and river margins in their larval stages and is a highly suitable taxa for Tier 2 testing in these regions. Yet little is known about how laboratory conditions affect sub-lethal endpoints such as emergence, larval stages and overall survival, resulting in difficulties with standardising testing. In the wild, individuals emerge across varied time periods and the emergence success rate and survival of larvae are largely unknown with many confounding factors contributing to growth and development.

Here, we present the results from non-standard bench-scale tests using wild-caught *C. dipterum* larvae. The larvae were subjected to different variations (from optimal to stress conditions) of environmental conditions, modifying temperature, photoperiod and food quantity. Data was collected for a range of developmental measurements, including body length, instar stage, feed intake, moults, time to emergence and survival were measured.

*C. dipterum* showed distinct trends in response to different conditions. In particular, sub-optimal temperature and food availability resulted in a significant impact on development and survival. Mortality increased with both low (16 °C) and high (22 and 24 °C) temperatures, and at the lowest food concentration. The increased mortality was mainly observed at instars L5 - L7. No clear trend was found for different photoperiods. A wider range of growth and development measurements, e.g., instar stages and body length was observed under optimum conditions (20 °C and higher food quantities) compared to the stress conditions. Ecologically speaking, variability in size and moults indicate different choices by individuals to invest in growth versus development which may have implications for population dynamics.

These results will be used to progress the reliability of mayfly testing in a regulatory context and inform future effect modelling.

#### **1.09.P-Mo080 Effects of the UV filter 4-hydroxybenzophenone at the molecular level on the aquatic invertebrate *Chironomus riparius***

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In recent years, UV filters have become ubiquitous environmental contaminants of increasing concern because of their extensive use, persistence, and bioaccumulation potential. The UV filter 4-hydroxybenzophenone (4HB) has been described to cause endocrine disruption in vertebrates and is found in natural aquatic environments. Here, the ability of aquatic invertebrates to manage the emerging contaminant 4HB is studied. To address this, the reference organism *Chironomus riparius* was in vivo exposed to three environmentally relevant concentrations of 4HB (0.1, 1 and 10 mg/L) for 8, 24 and 72 hours. The response was assessed by analyzing the differential expression of genes involved in detoxification, stress response, energy metabolism, drug resistance, and DNA damage repairing. The analysis was performed using a 16-gene real-time PCR array. The results suggest that exposure to 4HB stimulates the detoxification mechanisms and the toxicant export out of the cytoplasm causing an increase in the expression of the MRP1 transporter. Finally concluding that additional research is needed although the effects observed in this study suggest that it produces a weak response in the short-term.

#### **1.09.P-Mo081 Toxicology Effects of Guanidinium Isothiocyanate In the Biological Model: *Caenorhabditis Elegans***

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**Background:** Guanidinium isothiocyanate (GITC) is a chaotropic agent that is used as the active ingredient in lysis buffers in nucleic acid extraction processes and whose use increased in laboratories during the pandemic (1,2). It is currently identified in the Network for Monitoring and Evaluation of Emerging Chemical Substances (Norman Network) on the list of suspects for Emerging Contaminants (3). Its inclusion as a suspicious substance is a cause for concern considering that this reagent is for use in the laboratory and must be discarded under special conditions and in accordance with the danger categories for “substances dangerous for the aquatic environment” of the Economic Commission to the United Nations for Europe. GITC is classified in category: acute toxicity 3, being toxic to some fish and crustaceans but not classified as a substance that generates chronic toxicity (4,5).

**Objective:** To evaluate the toxic effects at different concentrations of GITC in the *Caenorhabditis elegans* biological model.

**Methodology:** GITC concentrations that caused mortality in wild strains (Bristol N2) of exposed *C. elegans* were identified, estimating the LC 50. Growth, locomotion, and reproduction parameters were evaluated according to the exposure and changes in expression were established of stress response genes in transgenic strains of *C. elegans*.

**Results:** the concentrations used did not induce acute lethality. There was alteration in growth, locomotion, and reproduction. There was an increase in the expression of genes related to oxidative stress.

**Conclusion:** GITC generated effects on locomotion, growth and reproduction in the nematode, and oxidative stress enzymes were also expressed.

### **1.09.P-Mo082 A geometric framework approach to understand multi-metal toxicity on individual organisms to evaluate relative risks and benefits of pollution and mitigation**

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The essential nature of organic metals to organismal fitness makes them distinct from other common pollutants. The interdependence of these metals in cellular function, uptake, and excretion, are probable to make their dose-responses interdependent, whilst also being dependant on other environmental and physiological conditions. The potential for less than additive or antagonistic effects of certain metal combinations could have important implications in mitigating the impacts of organic metal toxicity in the environment. However, we lack a comprehensive assessment of multi-metal interactions, with most of our knowledge limited to studies of single or binary combinations of essential metals, across a narrow set of doses. We aim to generate a multivariate geometric framework, across multiple organic metals, as an intuitive means of mapping the effects of expose on phenotypic outcomes. We use the fruit fly (*Drosophila melanogaster*), an ideal high throughput model to achieve such an extensive assessment of candidate metals. Initial focus of the study will be on developmental and reproductive endpoints in flies exposed to combinations of copper, zinc, and phosphorus; elements important in shaping organismal health, that are also environmentally relevant pollutants. The protocols developed from this study can then be applied to a more extensive range of candidate metals to identify those that are highly interactive. In addition to other benefits of using a short-lived invertebrate model, the fly genome is comprehensively understood and easily manipulatable, making this species a particularly effective tool to explore the largely unknown mechanisms underlying the physiological effects of the interactions between metals. For example, metal toxicity effects have been studied in detail in the fly with a key regulatory role of the transcription factor MTF-1. Using flies mutant for this transcription factor will allow us to distinguish dysregulation of metal homeostasis from direct effects of metals and their interactions. Our work aims to provide a more comprehensive understanding of how organic metals shape organismal health and fitness on a fundamental level.

### **1.09.P-Mo083 Effects of three antibiotics (tetracycline, ciprofloxacin, sulfamethoxazole) on survival, growth, reproduction, behaviour and antioxidant enzymes of the earthworm *Dendrobaena veneta***

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Antibiotics have been widely used in human and animal medicine and are identified as emerging aquatic and terrestrial contaminants. Veterinary antibiotics are used for disease prevention, control and treatment in food animal production. Worldwide demand for livestock production is expected to double by 2050 implying considerable use of antibiotics. A wide variety of antibiotics has been detected in aquatic and terrestrial environments, among which tetracyclines, sulfonamides and quinolones are the most frequently detected. Because of inherent biological activity antibiotics present in the soil may impact soil dwelling organisms, however their effects on soil dwelling organisms are still poorly understood. Adult earthworms *Dendrobaena veneta* were exposed to 1-500 mg/kg of tetracycline (TC), ciprofloxacin (CIP) or sulfamethoxazole (SMX) for 8 weeks. Mortality, body weight, cocoon production and biochemical responses including the activity of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione-S-transferase (GST) and malondialdehyde (MDA) were examined. During the avoidance test, the earthworms were exposed to the same antibiotics concentration in the soil for 48 hours. The results showed obvious avoidance behaviour of *D. veneta* against CIP and SMX, though no avoidance was observed against tetracycline. The survival of the earthworm was affected only at the highest tested antibiotic concentration and only after eight weeks of exposure. Only SMX significantly affected the final earthworm weight, however, the pattern of body weight growth of earthworms exposed to different antibiotics was dissimilar. SMX has also shown toxicity to cocoon production and juvenile hatching. The activity of antioxidant enzymes (catalase, superoxide dismutase and glutathione-S-transferase) was also affected by antibiotics.

### **1.09.P-Mo084 Assessment of Emerging Pollutants in Wastewater Impact on Fuente de Piedra Wetland: A Toxicological Study on a Native Species (*Daphnia magna*)**

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Wastewater discharges from local treatment plants in freshwater ecosystems are the main point source of emerging pollutants input into wetlands. The continuous input and presence of emerging pollutants like pesticides, stimulant and pharmaceutical compounds into freshwater and their potential effects on planktonic organisms are raising concern. Under this scenario, we investigated the presence of emerging pollutants in the Fuente de Piedra RAMSAR wetland (Málaga, Spain). We detected the presence of a total of 34 emerging pollutants in all water samples analysed over a period of six months. The most predominant contaminants were pharmaceuticals (13), followed by pesticides (11), drugs and stimulant substances (9), and personal care products (1). Among these, caffeine, the nonsteroidal anti-inflammatory drug naproxen and the neonicotinoid systemic insecticide imidacloprid showed the highest concentrations. Concentrations varied between 0.09 to 15.08 µg/L for caffeine and between nondetectable to 2.30 µg/L and 0.46 µg/L for naproxen and imidacloprid, respectively. According to the results obtained, we propose to carry out toxicological test to investigate their potential toxic effects on the native species from Fuente de Piedra *Daphnia magna*. Our approach is evaluating the potential ecological risk of caffeine, imidacloprid, and naproxen on *Daphnia magna* increasing concentrations (0, 0.1, 10, 100, 1,000, 10,000, 50,000, 100,000, 500,000 µg/L) and monitoring for acute effects. The concentrations selected cover a wide range to ensure a comprehensive evaluation of potential toxicity. Preliminary results indicate that caffeine, imidacloprid, and naproxen exhibit concentration-dependent effects on *Daphnia magna*, with immobilization as a primary endpoint.

The relevance of this study lies in its contribution to understanding the ecological consequences of wastewater-borne emerging pollutants on aquatic ecosystems. The findings provide valuable insights into the potential risks associated with specific pollutants, such as caffeine, imidacloprid, and naproxen, and their impact on *Daphnia magna*. This research provides insights into the adverse effects of emerging pollutants on planktonic native organisms for a better ecological risk assessment in contaminated areas. Subsequent efforts aimed at mitigating these effects should take our results into consideration.

#### **1.09.P-Mo085 Acute and chronic toxicity of four short- and ultrashort-chain perfluoroalkyl substances in *Daphnia magna* and *Hydra vulgaris***

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Perfluoroalkyl substances (PFAS) have been used for decades in industrial and commercial products and are detected worldwide in aquatic and terrestrial environments. Short substances (< 6C) such as perfluorobutanoic acid (PFBA), perfluorobutane sulfonic acid (PFBS), and trifluoroacetic acid (TFA) have been increasingly used by industry and are now detected in environmental matrices. We need to better understand the effects of these PFAS on aquatic organisms to be able to assess their risks. The first objective of this study was to investigate the acute toxicity of four short- and ultrashort PFAS - PFBS (4C), PFBA (4C), TFA (2C) and trifluoromethane sulfonic acid (TFMS, 1C) - in two aquatic invertebrate species: *Daphnia magna* and *Hydra vulgaris*. Two independent experiments were conducted with the same individual PFAS. In one experiment there was no pH adjustment of the solutions, whereas in the other experiment the test solutions were adjusted to pH of 6,5 to 8,5 corresponding to standard exposure media. The second objective was to assess the chronic toxicity of TFA in both species (treatments = 0, 0.01, 1, 10, 100 mg/L; 13 d exposure for *D. magna* and 7 d for *H. vulgaris*). Endpoints of immobility, mortality, reproduction, body size, and glutathion S-transférase (GST) as biomarker of oxidative stress were assessed in *D. magna* and development and reproduction were evaluated in *H. vulgaris* during and after exposure. Chemical analysis confirmed the presence and stability of the four PFAS in exposure media. Results from acute testing without pH adjustment (LC50 = 316 mg/L and 31,6 mg/L for *D. magna* and *H. vulgaris*, respectively) indicated that the four PFAS impacted the survival of both species at the highest concentration (1000 mg/L); results for all LC50 were > 1000 mg/L with pH adjustment. Results from chronic testing on *D. magna* indicated no effects on mortality, immobility, reproduction, and GST levels in comparison to controls. A size reduction was observed for *D. magna* with increasing concentrations for TFA during chronic testing. TFA exposure did not induce changes in the morphology of individual polyps and did not alter the reproduction of hydra. Overall, results suggest lower toxicity of these substances compared to longer-chain PFAS. However, the persistence of these short-chain PFAS in the environment and potential sub-lethal effects should be investigated.

#### **1.09.P-Mo086 A High Throughput Adaptation of Standardized Ecotoxicological Exposure Tests in *Daphnia magna* and Other Invertebrates**

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Modern mechanistic modelling can create extrapolations of chemical toxicity across multiple organisms; however, it tends to require large amounts of measurements. New technology and equipment have introduced the opportunity of using high-throughput testing techniques to facilitate the collection and automated processing of high volumes of experimental data assisted by Artificial Intelligence or specialized image analysis software.

The present project aims to adapt the *Daphnia magna* chronic OECD standard procedures to a High Throughput setting using a Cell imager and well plates. The method will then be extended to other invertebrates used in similar ecotoxicological tests,

such as *Moina macrocopa*, *Thamnocephalus platyurus* and *Heterocypris incongruens*. We aim to standardize high throughput methods, expose the organisms to different chemicals with varying modes of action and octanol-water partition coefficients, measure chronic toxicity responses and derive Dynamic Energy Budget Theory (DEB) Toxicokinetic – Toxicodynamic (TKTD) parameters.

In Initial experiments, *D. magna* was grown in ElenDt M7 media in 2ml well plates. Growth, defined as head to tail length, matched observed values in the literature where OECD standards were followed. Lengths ranged between 1.8-3.0 cm after 14 days, with little mortality (<20%). Follow up experiments will feature cohorts of 60 organisms exposed to five different concentrations of 3,4-Dichloroaniline, 2,4,5-Trichloroaniline, 1,4-Dichlorobenzene and 3-Nitroaniline for 21 days, quantifying effects on their growth and reproduction. Experimental concentrations for the selected chemicals will be derived after an initial experiment to create a dose-response curve and obtain an EC50. For the presentation, results for *M. macrocopa*, *T. platyurus* and *H. incongruens* will also be included.

The next step will also include the exposure and culturing of all the species under different temperatures, food regimes and chemicals, in order to generate growth curves and derive DEB parameters, such data are currently unavailable for *T. platyurus* and *H. incongruens* in the Add-my-pet database. After this, the data generated will be inputted into a DEB-TKTD model to predict chemical toxicity across multiple species and chemicals with varying physicochemical properties and modes of action. Finally, the predictions of the model will be fitted to new experimental data.

#### **1.09.P-Mo087 Nanoplastic-Induced Genotoxicity in *Daphnia pulex***

*Thomas Nash, Paul Kay and Laura Carter, School of Geography, University of Leeds, United Kingdom*

The presence of microplastics in freshwater systems are cause for concern, with many studies detailing harm caused to freshwater organisms resultant of exposure to microplastics at both an individual and community level. Exposure to microplastics is known to negatively impact daphnids, causing increased mortality and a range of sublethal effects including reduced reproduction and changes to swimming behaviour.

Genotoxicity testing can provide another useful endpoint in this regard since it can detect sublethal – but still potentially harmful – effects across varied timescales. Combined with other biomarkers, genotoxicity testing can improve understanding of the ecological consequences of environmental contamination.

In this study, *Daphnia pulex* neonates were exposed to 100nm polystyrene microparticles for 24 and 48 hours at doses ranging from 10 to 200 mg/L, with immobilisation and genotoxicity (comet assay) endpoints assessed following exposure. Results indicate that polystyrene microparticles at high concentrations induce genotoxicity within 48 hours, however immobilisation proved more sensitive to the presence of polystyrene microparticles at both lower concentrations and after just 24 hours. Further work will investigate the genotoxic impact of microplastics both in longer term (chronic) exposures and in combination with other pollutants.

#### **1.09.P-Mo088 "An automated flow-through exposure system for the evaluation of challenging substances in the *Daphnia* reproduction test"**

*Anne Dupont, Armin Peither, Séverin Semal, Johannes Völker, Stefan Josef Hoeger and Jörn Schreitmüller, Innovative Environmental Services (IES) Ltd., Switzerland*

The *Daphnia magna* reproduction test plays a crucial role in the regulation of chemical substances. The test involves exposing juvenile female *Daphnia* to the test substance over 21 days and assessing several reproductive parameters, including the number of offspring, time to the first brood, and the survival and growth (measured in length) of the parent organisms.

The frequency of medium renewal depends on the stability of the test substance. According to the test guidelines, medium renewal should be done at least three times a week. For challenging test substances that are volatile or tend to degrade quickly (such as hydrolysis, oxidation, and sorption), the OECD recommends more frequent medium renewal or the adoption of a flow-through test (OECD Guideline 211; OECD Guidance Document 23).

A flow-through exposure system enables the maintenance of stable exposure conditions and, thus, the assessment of difficult test substances. At IES, we use an automated flow-through dosing system with individual dosing units for each treatment. Based on the specific physicochemical properties of the test chemical, solubility tests and dosing pre-experiments are performed to determine and optimise appropriate dosing parameters including test medium flow and exchange rate as well as ensuring adequate nutrient supply.

Given the increasing demand for chronic toxicity testing of challenging test substances, the possibilities and various setting of the flow-through exposure system are presented, discussed, and compared with the semi-static approach. We will focus on the reproduction rate and the analytical results (recovery), as these two parameters are crucial for the acceptance of the test results for the risk assessment of biocides, plant protection products and industrial chemicals.

### 1.09.P-Mo089 JC-1 and High-Content Imaging to Quantify Mitochondrial Toxicity in *Daphnia magna*

*Cedric Abele, Amira Perez, Andrey Höglund, Paula Pierozan, Magnus Breitholtz and Oskar Karlsson, Environmental Science, Stockholm University, Sweden*

New approach methodologies are required to improve chemical risk assessment. Alternative endpoints are e.g. necessary to elucidate the toxic mechanism of a chemical towards organisms. Fluorescence microscopy and the development of molecular dyes offer new possibilities to detect changes in biochemical processes due to chemical stressors.

The aim of this study was to quantify the effect of the model compounds 2,4-Dinitrophenol (2,4-DNP) and carbonyl cyanide 3-chlorophenylhydrazone (CCCP) on mitochondria health in *Daphnia magna* by using the mitochondrial membrane potential dye JC-1 in combination with automated image-analysis.

*D. magna* were exposed to 2,4-DNP concentrations between 0.94 and 40 mg/L and CCCP between 0.5 and 2500 µg/L for 2 h and 24 h. Five daphnids per concentration were used and the experiments were conducted in triplicates. After the exposure, immobilization was measured and JC-1 was applied. Fluorescence images were acquired in an automated confocal high-content imaging system. The signal intensities in *D. magna* were measured using an established image analysis workflow. Dose-response relationships based on the change in fluorescence intensities were calculated.

In all experiments, effects on the mitochondrial membrane potential were detected at lower concentrations than immobilization. Moreover, the *D. magna* that showed altered JC-1 signal were fully mobile, showing that this effect is sublethal. In both experiments, the EC<sub>50</sub>-values of mitochondrial membrane potentials after 2 h exposure were similar to their corresponding EC<sub>50</sub>-values of immobilization after 24 h. After exposure to 2,4-DNP, mitochondrial effect concentration was three times lower compared to corresponding values for immobilization and up to 30 times lower after exposure to CCCP.

These results indicate that the image-based method is able to identify the difference in the test compounds' potency of uncoupling oxidative phosphorylation in mitochondria of *D. magna*. Furthermore, effects were measured at a much earlier stage and predicted organism death after 24h and 48h. In conclusion, this new approach provides an rapid and sensitive mechanistic approach to regular ecotoxicity testing of chemicals.

### 1.09.P-Mo090 The Effect of Wildland Fire-fighting Chemicals on the Reproductive Success of *Ceriodaphnia dubia*

*Holly J Puglis, Michael Iacchetta and Christina Mackey, U.S. Geological Survey (USGS)*

With wildfires increasing in frequency and intensity in the western United States, it is essential to understand how the chemicals used to suppress the spread of wildfire interact with the landscape. The effect on environmental health must be understood to use these chemicals safely and effectively in emergencies. Studies have recently been published on the lethality of fire-fighting chemicals currently used by the U.S. Forest Service; however, little is known about how these chemicals may impact the reproductive success of organisms affected by chemical intrusions. We investigated the reproductive success of a commonly tested cladoceran (*Ceriodaphnia dubia*). Specifically, female cladocerans were exposed to a concentration series of three current-use fire-fighting chemicals (LC95A-R, MVP-Fx, and 259-Fx) at two durations (15 or 60 minutes) and then held for seven days to determine effects on reproduction. There was a significant interaction between concentration and duration of exposure with a decrease in number of neonates produced per female in LC95A-R treatments after 60 minutes of exposure at 3,500 mg/L and greater concentrations compared to the control females. There was a similar pattern with MVP-Fx, although the interaction was not significant. In 259-Fx treatments, there was no interaction between duration of exposure and concentration, but fewer neonates were found in treatments with 60-minute exposures than in 15-minute exposure treatments. Measures of reproduction are a hallmark indicator of the ecological impact of chemical products on biota because impaired reproduction has significant consequences for the structure of natural populations, as well as for the stability of the food web. Further investigation of aquatic biota found in streams native to areas with high occurrence of wildfire should be studied to determine the potential population level changes that may occur from fire-chemical intrusion.

### 1.09.P-Mo091 Exploring mode-of-action for acute toxicity of primary aromatic amines with *D. magna* using differential gene expression analysis

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Organic pollutants, with their diverse structures and modes-of-action (MOAs), pose a significant challenge in understanding and predicting their toxic effects on the environment. Chemicals can be grouped according to toxicity in among others non-polar narcotics, polar narcotics, reactive chemicals, and specifically acting chemicals. Non-polar narcotics (baseline toxicants) act via a non-specific MOA, with their toxicity linked to hydrophobicity. Polar narcotics are slightly more toxic, while reactive and specifically acting chemicals show enhanced toxicity due to more specific MOAs and targets. The MOA of a chemical is also linked with its interspecies variation in toxicity: structurally analogous narcotics typically demonstrate limited interspecies variations in toxicity, while chemicals with a specific or reactive MOA display elevated interspecies variation, primarily attributed to the specific MOA in exceptionally sensitive species. In that respect, aromatic amines exhibit distinctive toxicity characteristics. Although they have been classified as 'polar narcotics', certain substitution patterns of the aromatically bound amino group result in a large interspecies variation in toxicity, due to their excess toxicity towards species such as *D. magna*. In this context, we investigated three primary aromatic amines (PAAs) in *D. magna*: 4,4'-MDA, 2,2'-MDA and 2,4-TDA.

Despite their structural and chemical similarities, they exert very distinct biological effects. Therefore, we studied their MOA by acute exposure experiments in the model organism *D. magna* to study its gene expression in response to PAA exposure. Our experimental design also included two reference compounds, a non-polar narcotic (1-octanol) and aniline. Our analysis showed, both in terms of acute immobility and gene expression patterns, very distinct biological MOAs for the three PAAs. While 4,4'-MDA treatment showed similar effects as aniline, its isomer 2,2'-MDA showed similar effects as 1-octanol. 2,4-TDA had an EC10 value in between the other two PAAs and did not induce any differential gene expression compared to the negative control at its EC10 concentration. These results indicate towards a specific influence of substituent positioning in PAAs on their toxicity and MOA towards *D. magna*.

#### **1.09.P-Mo092 Alternative Assays for Routine Toxicity Assessment**

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Substantial number of aquatic toxicity tests exist and are deployed in particular frameworks. However, for routine screening of environmental samples and potential chemical formulation investigations, these conventional assays are not practical and hinder expeditious progress. The development of alternative small-scale bioassays referred to as microbiotests has significantly advanced throughput strategies. The microbiotest technology has several advantages in comparison to the "traditional" tests based on laboratory cultures, especially its independence of the stock culturing burden.

The Daphtoxkit is a prime example of a microbiotest that has been effective and has enabled implementation of projects worldwide otherwise not doable and has accelerated the advancement of research & development programs. Microbiotests offer new opportunities for effects monitoring of chemicals and environmental matrices (effluents, leachates, interstitial waters, etc). These can also contribute to increasing the cost efficiency and diagnostic potential of hazard assessment schemes.

Use of microbiotests is continuing to gather momentum for application in regulatory frameworks. Aquatic microbiotests, invaluable for screening and ranking ecotoxic effects, their intrinsic features can contribute to novel applications enabling rapid detection and enhanced throughput.

Biological testing, and microbiotesting in particular, will indubitably increase significantly in environmental protection activities in the future. International recognition of biotesting, and more critically owing to limitations in regional testing infrastructure (lack of laboratories and culturing facilities), the implementation scope is globally significantly enhanced to facilitate environmental policies, regulations and guidelines. The imperative need for cost efficiency in environmental assessment continues to fuel the need for R & D concerning microbiotesting.

#### **1.09.P-Mo093 Protection over Prediction: Daphnids Safeguard the Use of Alternatives to the Acute Fish Toxicity Test**

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The assessment of potential environmental impacts of chemicals traditionally involves acute aquatic toxicity tests using algae, daphnids, and juvenile fish, representing different trophic levels. As part of the international 3R goals, alternative tests like the acute fish toxicity test with the fish cell line RTgill-W1 and the zebrafish embryo acute toxicity test were developed to replace the juvenile fish test, which is in line with the European Chemical Strategy for Sustainability. A related OECD project to develop Integrated Approaches to Testing and Assessment (IATAs) for acute fish toxicity is ongoing.

Previous studies revealed the lower sensitivity of these alternative tests for some neurotoxic chemicals and allyl alcohol, which is biotransformed to the more toxic acrolein in fish. We analyzed historic toxicity data from the EnviroTox Database and found considerable variability in acute fish LC50s and acute daphnids EC50s, particularly for neurotoxic chemicals. Comparing sensitivity of these taxonomic groups according to different neurotoxicity classification schemes indicates that fish rarely represent the most sensitive trophic level, especially among chemicals with neurotoxic modes of action, except for a few cyclodienes, which are no longer relevant on the market and could be identified through structural alerts. Moreover, daphnids were more sensitive than fish for allyl alcohol toxicity. This analysis highlights the potential of the standard *Daphnia* acute toxicity test guideline in safeguarding the environmental protection level provided by alternative methods.

This research, rooted in decades of efforts to replace the juvenile acute fish toxicity test, expands the focus from predicting fish toxicity to emphasizing environmental protection. This broader perspective enables the complete replacement of the juvenile fish test within an IATA, standardized by the OECD. Furthermore, it paves the way for further eliminating vertebrate tests in environmental toxicology.

#### **1.09.P-Mo094 Inorganic turbidity modifies the effect of glyphosate on *Daphnia magna***

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Nowadays, glyphosate is the most widely used post-emergent herbicide in the world. Although it is stated that the ecological

risks of glyphosate on non-target species are minimal, the potential carcinogenic effect of this pesticide on humans has been demonstrated, as well as its toxic effects on aquatic biota. This situation has led to its use being banned in several countries. On the other hand, organic turbidity related to soil erosion due to deforestation and unsuitable agricultural practices contributes to an increase in inorganic turbidity in the aquatic environment, which affects non-selective filter-feeding zooplankton, such as cladocerans. In this study, the effect of the presence of clays (40 mg L<sup>-1</sup>) on the acute and chronic toxicity (21 d) of the Faena® glyphosate formulation in *Daphnia magna* was evaluated. The sublethal concentrations assessed were 2, 4, and 6 mg L<sup>-1</sup> as glyphosate. The presence of clay slightly reduced the acute (48-h) toxic effect (LC<sub>50</sub>= 8.79 vs. LC<sub>50</sub>=9.69 mg L<sup>-1</sup>, respectively). For chronic exposure, the three concentrations of Faena® reduced accumulated progeny. When clay was added, the fecundity of *D. magna* was only reduced at the concentration of 6 mg L<sup>-1</sup>. In controls, clay reduced total progeny. The number of clutches without clay was only lower at the highest concentration of glyphosate, and there were no differences at any concentration when clay was added. The age of first reproduction was merely delayed by clay in the control and the concentration of 2 mg L<sup>-1</sup>. In the glyphosate-only assays, there was an increasing number of abortions in direct relation to the concentration. In contrast, in the clay experiments, abortions were only recorded in the control and the glyphosate concentration of 6 mg L<sup>-1</sup>. Mortality was only significantly higher in glyphosate 6 mg L<sup>-1</sup> with no clay. The results indicate a reduction in the toxic effects of the Faena® glyphosate formulation on *D. magna*, which could be due to a possible retention effect due to the mineral clay charges on some toxic ingredients of the formulation Faena®, in addition to glyphosate. However, it should be noted that both the toxicity of chemical pollution and the increase in inorganic solids in the aquatic environment produce adverse effects on the biota, in addition to affecting essential processes such as photosynthesis due to the interference with light by the increase in turbidity, and effects on filtration rates of zooplankton and benthic organisms.

#### **1.09.P-Mo095 Prediction of the toxicity of 14 compounds on *Daphnia magna* by using cultures of the yeast *Schizosaccharomyces pombe***

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The aim of this work was to compare the toxicities on the wild strain of *S. pombe* and *D. magna* to compare the sensitivity and correlation among each experimental models. For all the compounds tested, the toxicity decreased in the following order: triclosan, carbendazim, propylparaben, rotenone, paraquat, chloroquine diphosphate, hydrogen peroxide, perfluorooctanoic acid, hydroxyurea, dibutylphthalate, carbamazepine, metoclopramide, melatonin and KCl.

Although *D. magna* is more sensitive than *S. pombe*, the correlation between the EC50 values obtained for the 14 compounds in the wild strain of *S. pombe* at 20h and for the crustacean *D. magna* exposed for 48h was quite good (R<sup>2</sup>=0.9282). Therefore, taking into account the limitations due to the small number of compounds tested, *S. pombe* allows the prediction of the range of toxicity of the substances to *D. magna*, and, viceversa.

It should be noted that the compound that is furthest from the trend line was rotenone, which presented much lower toxicity for *S. pombe* than for *D. magna*. This fact is understandable, since fission yeast does not have the main target of the toxic action of the pesticide, that is, complex I. Furthermore, short term *S. pombe* cultures can be applied to predict the range of toxicity on *D. magna*.

#### **1.09.P-Mo096 Assessing the Toxicity of Cytarabine in *Daphnia magna* Through Apical and Cellular Endpoints**

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Due to the growing number of cancer cases, the occurrence of antineoplastic agents (AAs) in the environment is increasing, posing a significant threat to aquatic ecosystems. The primary sources of AAs' entry into the environment include hospital effluents, household discharges, and pharmaceutical manufacturing. Due to inadequate removal during wastewater treatment plant, AAs persist in surface, ground, and drinking water. AAs are one of the least studied groups concerning environmental impact and have been considered to represent specific risks to non-target aquatic species due to their mode of action. The aim of this study is to assess the effects of cytarabine (CYT) on the survival and reproduction output as well as analyze the biochemical alterations that it may induce to *Daphnia magna*. CYT is an AA that disrupts the DNA replication process, inhibiting the synthesis of deoxyribonucleic acid (DNA) in cells. To achieve this, *D. magna* individuals were exposed to a range of concentrations according to the OECD 202 and 210 guidelines and effects on their survival and reproduction were evaluated. For a more comprehensive understanding of the toxic effects of CYT, the activity of catalase (CAT), glutathione S-transferase (GST), acetylcholinesterase (AChE), and lipid peroxidation (LPO) were measured after 21 days of exposure. The results from the acute exposure showed no mortality at concentrations of up to 100 mg/L, while the reproduction test demonstrated a significant reduction in the offspring of *D. magna* across nearly all tested concentrations with the lowest concentration the exception. A 21 d EC<sub>50</sub> of 20.65 ± 1.51 mg/L was obtained, with the highest concentration promoting almost a total inhibition to the offspring output. Regarding the biochemical determinations, no significant alterations were observed for CAT, GST and LPO, however, for AChE a decrease in activity was observed with significant differences at one of the concentrations tested. The results obtained in this study indicate that, despite not being able to induce alterations in short-term exposures, CYT can negatively impact the reproductive system of *D. magna* and compromise its offspring. Even though the

chronic ecotoxicological data presented in this study highlight the potential risk associated with AAs, effects are observed at concentrations that are not environmentally realistic.

#### **1.09.P-Mo097 Model species culturing made easier using an automated device: case study *D. magna***

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The pollution of environmental components is being characterised by the appearance of new toxicants, multicomponent emissions and discharges, pollutants speciation in the air, water and soil, or other complex processes. Bioassays allow to determine the integral toxicity of the studied environment and extrapolate the data obtained to real ecosystems. To optimize results, it is necessary to consider the sensitivity of the biological species to toxicants, the adaptive capabilities of organisms in the environment, as well as the action of a complex of abiotic (temperature of cultivated water, its chemical composition, season of the year) and biotic factors (such as seeding density). The need to combine chemical methods and bioassays is a generally recognised trend in planning environmental studies. One of the key points to optimisation resides on the culture of model species, where standardisation of the conditions for test organisms have been promoted; however, routine monitoring of cultures requires effort and time.

This work aims to evaluate an automated computerised device assisting culture monitoring of model species, leading to easy routine monitoring. The work presents a case study using *Daphnia magna*, and considering some life history traits of *D. magna*, such as survival time, body length at maturation, and offspring per female.

The counting of neonate production can be easily achieved by using automated device, contrary to what is required by manual counting. Some other life history traits, such as time to maturation, time to first brood, and time to next brood, are also evaluated using the automated computer device. The discussed approach can be applied assisting routine monitoring model species demographic characteristics, complementing already implemented procedures of test-cultures standardisation, selecting organisms of similar size, saving time, avoiding human error, and improving culture synchronisation, by predicting the time when offsprings might occur.

#### **1.09.P-Mo098 *Daphnia magna* as an Indicator for Aquatic Species in the Non-target Risk Assessment of Genetically Modified Maize**

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Similar to pesticides, genetically modified (GM) GM crops are regulated worldwide and environmental risk assessment is one requirement for environmental release. As a surrogate for aquatic species, non-target tests of GM crops often include *Daphnia magna*. Developing suitable test protocols for GM plants and *D. magna* has been challenging, because plant material needs to be ingested by the test organisms (oral mode of action). We here present laboratory studies with insect-resistant GM maize producing multiple Cry proteins from the bacterium *Bacillus thuringiensis*. Our experiments address several difficulties with such studies, including the suitability of crop plant material as food for *D. magna*, the separation of Bt protein-effects from plant background effects, and the interpretation of effects in the context of biological relevance and natural range of variation. *Daphnia magna* can survive, grow and reproduce when fed only maize materials, although the performance was poorer than when fed algae, which indicates nutritional stress. Adverse effects were observed for Bt maize flour, originating from different production fields and years, but not for leaves or pollen, produced from plants grown concurrently in the glasshouse. Because leaves contained eight to ten times more Cry protein than flour, the effects of the flour were probably not caused by the Cry proteins, but by compositional differences between the plant backgrounds. Larger differences in life table and population parameters of *D. magna* were observed among 5 different non-GM maize lines, which were tested in a similar way to obtain a natural range of variation for each parameter. The protocols established for testing GM crops on *D. magna* could also be adapted for other orally active substances including, e.g., systemic or RNAi-based pesticides.

#### **1.09.P-Mo099 Dearbhla, the *Daphnia*, and her research highlights**

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*Daphnia* are a fantastic model for research, but also for research communication. The *Daphnia* Detectives project, was developed to communicate our research findings on the impacts of microplastics in freshwater environments using *Daphnia* as a model organism. The project consists of a bank of open access online resources that cover the topic of microplastics and *Daphnia*, from experimental design stages through to example results and data sets that can be used to explore data analysis themes within the high school curriculum context.

A pillowcase, some pegs and pink particles form an integral element of the *Daphnia* detective's activities, and are a good example of how every day and specialist items can be blended to fit science communication needs. Some of the key lessons from this project was to have a range in the level of detail and complexity, as not all schools or groups have the same baseline level of understanding. Through engaging the pupils, it also highlighted aspects of the research that we don't give a second thought to, such as the ethical consideration of research designs. Although this is a standard part of the research process, it is



refreshing to reconsider and evaluate the developments in this area, demonstrating that science communication and public engagement isn't just disseminating information but is a valuable experience for researchers too.

The importance and use of model organisms with research communication can further be supported through the Dearbhla Daphnia mascot, which showcases an interactive and creative way to quite literally bring Daphnia into the public domain and spark discussions on the importance of water quality, moving beyond an anthropocentric view to explore the ecosystem level consequences of this in an engaging and novel way. A unique mascot design, and project approach, facilitates discussions beyond those which would conventionally take place within Daphnia, or environmental, research and can bring this research and the big challenges being explored to audience which perhaps would not have traditionally engaged. This project showcases a range of medium and platforms focussed on Daphnia, that can be used to disseminate but also engage people in research from the conception of projects and experimental designs, which could be of interest to other people working with the Daphnia domain.

#### **1.09.P-Mo100 Development of a Transcriptomics-based Testing System to Identify Environmental Stressors in Freshwater Invertebrates using *Daphnia pulex* as a Model Organism**

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Chemical pollutants alone and as part of complex mixtures are a key challenge for aquatic freshwater ecosystems, especially when they occur in combination with other biotic or abiotic stressors. Freshwater catchments often receive complex mixtures of chemicals, including pesticides associated with farming activities. Assessing the impact that these chemicals may have on aquatic organisms continues to be challenging and this knowledge gap is particularly notorious for aquatic invertebrates.

In collaboration with the Environmental Agency in the UK, we set out to develop a novel approach to assess the potential adverse effects that chemicals associated with farmland catchments may have on freshwater invertebrates. To do so, we selected *Daphnia pulex* as a model species given its widespread range in the UK and across many temperate regions worldwide, the ease of culture in the laboratory and the genomic resources available. We set out to use whole body transcriptomics to assess the molecular effects of a selection of pesticides of concern both individually and as part of complex mixtures. This information will then be utilised to design a transcriptomics based assay capable of detecting low levels of stress following chemical exposures in this species. This methodology will function as an early warning system for adverse effects of chemical contamination in freshwater ecosystems and support management and conservation decisions in the future.

#### **1.09.P-Mo101 Long-term Consequences of a Mosquito Control Agent on Insect Emergence – Insights From a Four Year Mesocosm Study**

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Specialized riparian predators rely mostly on aquatic derived food. Changes in the emergence pattern of aquatic insects may thus have substantial consequences on prey availability for these predators with potential risks for associated terrestrial foodwebs through bottom-up and top-down regulation. Bti (*Bacillus thuringiensis* var. *israelensis*), a commonly used non-chemical pesticide applied to control mosquito outbreaks in floodplain areas, is known to also affect non-target insects including Chironomidae. This effect is particularly concerning as Chironomidae can contribute up to 90% to emergence in some ecosystems. To understand the long-term consequences of Bti exposure we employed the so-called floodplain pond mesocosm (FPM) system monitoring insect emergence over the course of 4 years. During these 4 years, we collected emerging insects using emergence traps from the ponds with Bti application at field relevant levels, although using different application intervals, being the independent factor (stressor). Emergence was collected between April and August starting in 2020 until 2023. Equal seasons of the investigated years are compared to understand long-term consequences of varying application intervals. While most samples are still under evaluation, data from the first year of exposure uncovered a shift of the peak emergence by 10 days combined with a 25% reduction in Bti treated ponds. We expect to see a habituation of emergence dynamics in the second and third year where treatment intervals are kept similar to the first year followed by a stronger reduction on the emergence of Chironomidae with shorter treatment intervals.

#### **1.09.P-Mo102 Beyond Microplastics: Water Soluble Synthetic Polymers Exert Sublethal Adverse Effects In The Freshwater Cladoceran *Daphnia magna***

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Plastic contamination can be considered a driver of global change. Nevertheless, polymer contamination is not limited exclusively to microplastic (MP). Water soluble polymers (WSP) are in fact a class of highly employed compounds whose prevalence and impact have been neglected until recent years. WSP are used in several industrial, dietary, domestic and medical products and are often applied for waste water treatment. Despite they cannot be seen washing up on shores like MP do, WSP are found to be present in the environment and hence can potentially interact with the biota. In this work we exposed the freshwater cladoceran *Daphnia magna* to five commonly used WSP. For our investigations we relied upon a 48h acute

toxicity test and a 21d chronic toxicity test at the end of which we analyzed the levels of reactive oxygen species and life history parameters. Furthermore, we developed a heart rate acute assay to assess any early stage physiological alteration. Our results highlight that the employed WSP do not exert any acute effect, however they did induce life history alterations together with an altered oxidative status. The recorded effects not only can potentially alter *D. magna*'s population dynamics, and thereby, given this species relevancy in lentic ecosystems, impact also higher trophic levels and the biological community. Therefore, further investigations on WSP's effects are highly desirable and their inclusion in hazard assessment regulations is urgently needed.

### **1.09.P-Mo103 The effects of Dexmedetomidine on the behaviour of crustaceans: lab to field approach**

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Dexmedetomidine is an  $\alpha_2$ -adrenergic receptors agonist and was originally authorised as a human/veterinary sedative but is now an active ingredient in antifouling paint for boats. In crustaceans, it is known to bind to octopamine receptors, which like other neurohormones, cascade to control many biological functions including behaviour. The aim of this study was to determine whether exposure to Dexmedetomidine can alter the behaviour of marine amphipods (*Echinogammarus marinus*) and juvenile crabs (*Carcinus maenas*) under laboratory conditions using both Zantiks and Daniovision behavioural analysis. Amphipod specimens were exposed to concentrations between 0.1 and 100 $\mu$ g/L with behaviour recorded at 24h, 72h, 96h, 7d, and 14d of exposure and compared to controls and octopamine exposed individuals. Exposure significantly increased activity and distance travelled of individuals compared to controls in both amphipods and juvenile crabs, with an interesting non-monotonic concentration effect observed. Using acoustic telemetry, we then set out to determine whether these results could be replicated in the field to test how effects recorded in the laboratory may relate to in situ ecology. To do so, adult *C. maenas* crabs were sampled in Portsmouth, UK and gradually acclimated to 24‰ salinity before being exposed 1 and 100 $\mu$ g/L of Dexmedetomidine and 1 $\mu$ g/L of octopamine and serotonin. Individuals were tagged using V5 180Khz tags and released into a saline lake, where their position was estimated using four HR2 receivers (Innovasea) deployed 200 meters apart. Preliminary results indicate that the distance travelled by adult shore crabs was increased for individuals exposed to Dexmedetomidine in the first 96 hours, effects consistent with those observed in laboratory experiments performed on juveniles *C. maenas* crabs and *E. marinus* amphipods. Increased activity was persistent over the 12-week in situ period, for individuals exposed to the highest concentration of 100 $\mu$ g/L. Increased activity was also translated in an increase of home range area used by *C. maenas* exposed to 100 $\mu$ g/L of dexmedetomidine. Further results will aim to determine whether exposure to Dexmedetomidine may affect other aspects of their ecology, such as their mortality and diurnal rhythms. The field experiment represents the first acoustic telemetry project with crabs, looking into the behavioural effects of an environmental contaminant.

### **1.10.A Legacy and Emerging Contaminants in Wildlife: Recent Advancements in Ecotoxicology and Risk Assessment**

#### **1.10.A.T-01 Per- and polyfluoroalkyl substances levels and effects in Finnish Waterbirds**

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The increased presence of endocrine disrupting chemicals (EDCs) in our environment poses a significant threat to wildlife. In the Baltic region, the common eider (*Somateria mollissima*) and the common goldeneye (*Bucephala clangula*) breeding populations have been declining in recent decades. While predation plays a significant role in this decline, other stressors may also contribute to this process. Notably, there is a growing sex ratio bias towards males in the adult populations of these 2 species as in several other species, high hatching failure in monitored eider colonies and general decline in the breeding success of both species. These observations have raised the question of whether EDCs might be involved in these processes.

We investigated the presence of 41 Per- and polyfluoroalkyl substances (PFAS) among other EDCs in eider (plasma and eggs) and goldeneye (plasma). Our research confirms the presence of a variety of PFAS in breeding females of both species. We are currently assessing if the PFAS exposure is associated with thyroid hormone levels; results will be presented at SETAC. We further assessed the link between PFAS and Avian Influenza Virus (AIV) occurrence in breeding females. Among the 45 sampled individuals, 75% were positive to AIV. Preliminary results did not show a relationship between PFAS exposure and presence of antibodies for AIV. We demonstrated a transfer of PFAS from female eiders to their eggs (18 PFAS among the 41 tested). In addition we have uncovered a positive correlation between the sum of 5 PFAS, among which perfluorododecane sulfonic acid (PFDoDS), and androstenedione (androgen and sex hormone precursor) in eggs with female embryos raising

concern on PFAS endocrine disrupting effects on female embryos. Following these results, an *in ovo* injection experiment with perfluoro-4-ethylcyclohexane sulfonic acid (PFECHS), PFDoDS and perfluorooctane sulfonic acid (PFOS) was carried out at NTNU, Norway with farmed mallard (*Anas platyrhynchos*) eggs. Results from different oxidative stress endpoints will be presented.

Our findings underscore the need for further studies on PFAS in waterbirds and their population level impacts.

#### **1.10.A.T-02 Urban Stressors on Avian Immune Defenses: Impact of pollution on Viral challenge via Poly I:C in great tit and blue tit nestlings**

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Rapid urbanization driven by human population growth alters landscapes, increases emissions, and disrupts habitats. This affects biodiversity, especially in birds adapted to city life, where chemical exposure is an indirect outcome. Most physiological research often focuses on human and poultry interactions, leaving gaps in understanding of how free-living birds respond to environmental chemicals. Urban areas have elevated air pollution, encompassing ozone, metals, nitrogen oxides, and particulate matter. Exposure to which induces oxidative stress, disrupting cellular balance. The innate and acquired immune system, regulated by antioxidant systems, becomes susceptible to oxidative stress. While most studies showed baseline immune parameters impacted in urban areas, few employ standard approaches, like immune challenges, to assess pollution's impact on pathogen response. Notably, studies of this nature used LPS and to our knowledge no study has explored how urban birds respond to viral challenges. Given the rising rates of avian influenza, understanding how pollutants affect birds' ability to establish anti-viral responses is crucial. To address this, we conducted a field experiment on great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) breeding in polluted (Rhenen) and clean (Wageningen) sites in the Netherlands. Passive NO<sub>2</sub> samplers were installed at each box. On days 8 and 15 post-hatch, mass, tarsus, and wing were recorded to estimate body condition. On day 13, four chicks within each box were selected, with two receiving a Poly I:C injection, and two a saline as control. Two days later, a blood sample was extracted, and the four chicks were euthanized for further analysis. Immune parameters, including haptoglobin (acute phase protein), immune cell counts, and Poly I:C specific gene expression (TLR3, RIG-1, IL-6, & IRF-7) were assessed and related to NO<sub>2</sub> levels. Preliminary results suggest that the atmospheric NO<sub>2</sub> levels were four fold higher in polluted site compared to the clean site and the NO<sub>2</sub> was positively correlated to baseline haptoglobin. However, when challenged with Poly I:C, nestlings in clean site exhibited a more robust immune response, with increased haptoglobin and higher immune cell counts compared to nestlings challenged in urban site. Additional analyses are pending, including the assessment of internal heavy metal concentrations and the incorporation of immune gene expression to explore the mechanistic pathways of Poly I:C.

#### **1.10.A.T-03 Quantify Exposure Levels of Non-persistent Chemicals in Wildlife from Tissue Concentrations, by Reverse Dosimetry Modelling using PBK-Models**

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Wildlife is exposed to different chemicals in the environment. To assess risks that such chemicals may pose, it is essential to have quantified insight in exposures. Monitoring of exposures in wildlife can be based on e.g., non-destructively collected samples or by using samples obtained from animals found dead. Historically, this has focused on legacy chemicals, including POPs for which interpretation of tissue concentrations is relatively straightforward. Their concentrations are directly related to (historic) uptake and often increase over time. Therefore, measured concentrations in tissues cover current and historic exposures and hence, risks. However, Contaminants of Emerging Concern (CECs) include chemicals that are less persistent, which can be metabolized by wildlife. For these, concentrations in tissues may decrease over time, making interpretation of (historic) exposure levels based on tissue concentrations difficult, often resulting in underestimation of risks. For such chemicals tissue concentrations are depending not only on levels of exposure but also on the capacity of the organism to metabolise it and the time between collection of the samples and the actual exposure. Physiological Based Kinetic (PBK-) models have been developed to quantify tissue concentrations of chemicals in organisms, based on exposure levels. In the current paper we present PBK-models for non-persistent pesticides, imidacloprid and carbendazim, for mouse (*Mus musculus*) and rat (*Rattus norvegicus*), and we will discuss the need for species specific and chemical specific rate constants for uptake and metabolism. These models include phase I metabolism, both total depletion of the parent compound as well as the formation of major phase I metabolites, and also the depletion of the phase-I metabolite, by phase II metabolism. This allows to quantify time-trends of concentrations of both parent compound as well as major metabolites. We will use these PBK-models for an innovative reverse dosimetry approach, which can be used to quantify oral exposures based on internal tissue concentrations. For this, the time between exposure and sample collection will be assessed based on the ratios of the concentrations of parent compound/metabolites, which increase over time, and can be used to base the reverse modelling of the exposures. We will apply the approach on experimentally obtained data for wood mouse (*Apodemus sylvaticus*) and discuss the applicability of this new approach.

#### **1.10.A.T-04 EXPOSO-METER: Characterizing the lifelong exposure (exposome) to environmental mixtures of pollutants at high trophic levels in Arctic marine mammals**

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EXPOSO-METER aims to provide access to the exposome by offering practical, economical and easy-to-use *chemometers* that allow for the identification, characterization and direct comparison of mixtures of environmental pollutants from multiple organisms, species and matrices. The *chemometer* approach is based on passive equilibrium sampling, and EXPOSO-METER aims to combine *chemometers* with advanced chemical profiling to integrate the lifelong exposure of environmental organisms to mixtures of pollutants. As *chemometers* are defined as a common reference phase, the chemical concentrations in the *chemometers* at equilibrium can be directly compared between organisms, circumventing normalizations. In this work, silicone *chemometers* have been used to analyze legacy and emerging hydrophobic organic pollutants (HOCs) present in 84 lipid-rich tissues from Arctic marine mammals collected in the surroundings of Tasiilaq and Kulusuk, at the southeast coast of Greenland. Samples of pilot whales, white-sided dolphins, white-beaked dolphins, narwhales, orcas and polar bears were analyzed. Circa 100 compounds were quantified (by gas chromatography coupled to high-resolution mass spectrometry), including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), pyrethroids (PYRs), chlorinated hydrocarbons (CHCs), organochlorine pesticides (OCPs), musk compounds, UV filters and antioxidants, and sex- and size/age-related patterns and intraspecies differences of contaminant exposure were investigated.

The results indicate that considering the fraction of each group of compounds in the total mixture, PCBs and OCPs were there predominant compound groups in all species, showing large variety between species, e.g. PCBs ranging from 35% in white-beaked dolphins to 68% in polar bears. Other groups of chemicals, like musk compounds, PAHs or PYRs show largely differing percentages in the different species that ranged across 2 orders of magnitude. The results also indicate interspecies differences in contaminant exposure, but no clear intraspecies differences, evaluated as sex- or size-related patterns. Within this project, *chemometers* will help to capture and understand the lifelong exposure of environmental organisms to mixtures of pollutants to strengthen the study of the exposome.

## **1.10.B Legacy and Emerging Contaminants in Wildlife: Recent Advancements in Ecotoxicology and Risk Assessment**

### **1.10.B.T-01 Pesticide contamination in bats: a case study on Greater Mouse-eared Bat in an agricultural and natural area in Southern Poland**

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Agricultural production across the world is highly dependent on the use of pesticides, which can be extremely dangerous to non-target arthropods, and especially to predators, which feed on insects, such as bats. According to the legal regulations in the EU, farmers are obliged to spray insecticides exclusively after sunset to limit the hazard to bees and other pollinators on the flowering cultures (e.g. oil-seed rape), which is the time when bats start to forage, potentially exposing them to high contamination risk.

The primary ways of poisoning bats with pesticides are the ingestion of contaminated food and water, inhalation and dermal contact. Taking into account that the activity of bats coincides with the recommended insecticide application time (evening and night) and considering the extremely high metabolic rate of bats as well as the large surface area of the wing membrane, we wanted to check if bats are directly exposed to pesticides.

To answer this question, we collected samples of insects and bat fur in two study areas – agricultural and natural ones in Southern Poland during May and June 2022 and 2023. Additionally, 15 carcasses of bats were collected for analysis from the agricultural area for pesticide screening in internal organs, such as kidneys and liver and also wing membrane. An agricultural area, where pesticides are regularly applied, was Ślężany village with a summer roosting colony of greater mouse-eared bat (*Myotis myotis*) in the school building. Our control site was in Sokole Góry nature reserve, where a large colony of *M. myotis* inhabits the Studnisko Cave. We used chromatographic analysis (GC/MS and HPLC/MS) to screen bat fur, wing membrane, kidneys and the liver and *Carabidae* beetles for the presence of pesticides.

We found in bat tissues and beetles 42 pesticides among 423 substances screened of which 23 were fungicides; 17 – insecticides and 2 were herbicides. Among these, 4 substances are currently banned in the EU: hexachlorobenzene, dieldrin, DDT (DDE, DDD) and chlorpyrifos. DDT and its metabolites (DDD and DDE) were present in bats and carabids from both the agricultural and natural areas, confirming the extremely long residence time of these compounds in the environment. Fortunately, in a comparison with concentrations found by other researchers in insectivorous bats which were poisoned by pesticides, the concentrations found in our study were lower and probably did not affect bat survival.

### **1.10.B.T-02 Calibration of mechanistic effect models for amphibians - challenges and solutions**

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Mechanistic effect models (MEMs) serve multiple useful purposes in a holistic risk assessment (RA) of chemicals to amphibian populations. A pivotal step in the development of MEMs is the development of physiological baseline models, which will have a large impact on the accuracy of predicted chemical effects. Generic models, such as Dynamic Energy

Budget (DEB) models, require adaptations to account for the specific life-history of amphibians. These deviations introduce difficulties in the estimation of DEB parameters from life table-data. Without manual intervention, common calibration routines often fail to correctly predict the timing of life stage transitions. We therefore provide an approach to calibrate mechanistic effect models, specifically developed for amphibian models. This approach is designed to minimize the necessity for manual intervention by modelling experts during the calibration, propagate uncertainties of parameter estimates and allow for varying levels of detail in the calibration data. We implemented a model based on Dynamic Energy Budget (DEB) theory, including adaptations to account for amphibian physiology. The calibration scheme progresses in multiple steps, where the starting point is a set of correlated probability distributions of DEB parameters which match the distributions of central physiology and life-history characteristics across a variety of amphibian species. This is followed by multiple parameter estimations, dealing successively with different life stages and transitions between life stages. Through the use of Approximate Bayesian Computation, the parameter uncertainty is propagated throughout the parameter estimation scheme. This scheme was applied in a case study using available *Xenopus laevis* growth data and resulted in a good fit to the data, including the timing of metamorphosis. Completing the calibration for a few data-rich species allows to use the gathered information to perform calibrations for data-poor species. At the minimum, estimates of age and body mass at life stage transitions are needed to perform parameter corrections for data-poor species. The approach will further be extended to the calibration of toxicity submodels, yielding whole life-cycle models which can be used to address risk assessment questions. This increases the applicability of the calibration approach to a wide range of species, helping to improve the environmental relevance of mechanistic effect models in the application of RA.

#### **1.10.B.T-03 Selenium accumulation and effects on freshwater turtle species from Tablas de Daimiel National Park (Spain)**

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The Tablas de Daimiel National Park (TDNP) is a Mediterranean alluvial plain formed by Cigüela and Guadiana rivers, located in the semi-arid region of central-southern Spain. It is declared a Biosphere Reserve, listed as a Wetland of International Importance by the Ramsar Convention and belongs to the European Natura 2000 Network. The wetland is threatened by the overexploitation of freshwater resources, aggravated by climate change. Pollutants reach the wetland through water seepage from surrounding agricultural lands and upstream WWTP discharges. Thus, high concentration of metals and metalloids of anthropogenic origin could be expected in the Park, as shown by previous studies detecting high levels of Se in sediments and biota. Although Se is an essential element due to its antioxidant properties, at toxic levels it interferes with sulfur-containing molecules and causes different effects like reproductive failure or abnormalities in the tegumentary. We hypothesize that freshwater turtles from TDNP affected by increased Se accumulation could suffer shell malformations because of the high proportion of sulfur-rich amino acids in the keratin covering the carapace. In July 2018, we captured 6 European pond turtles (*Emys orbicularis*). In October 2023, we captured 18 Iberian pond turtles (*Mauremys leprosa*) from the area within the Park with highest Se levels. All individuals were sexed, weighed and measured for shell curvature, and 1 mL blood was taken from the occipital sinus together with a sample of carapace scales to determine Se levels by ICP-MS. High incidence of *E. orbicularis* individuals with keratinization abnormalities and loss of shell keratin plates was detected. Turtles showing these abnormalities had close-to-significantly higher levels of Se than unaffected ones in both blood ( $p=0.06$ ). Se levels in blood and scales were correlated ( $p=0.036$ ). Our results suggest that Se exposure could be the reason for the observed abnormalities and allow us to determine that the malformations in *E. orbicularis* carapace are susceptible to occur blood Se levels above 6.1 mg/kg d.w.

#### **1.10.B.T-04 ELSATA: Update on a Thyroid-Focused Alternative to the Larval Amphibian Growth and Development Assay (LAGDA)**

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The Larval Amphibian Growth and Development Assay (LAGDA, OECD TG 241) was designed as a higher tier in vivo assay meant to provide data on adverse effects of substances on thyroid-mediated endpoints. As a level 4 test within the OECD Conceptual Framework on Endocrine Disrupters Testing and Assessment, it is a final step of testing in the ECHA-EFSA testing scheme for the thyroid modality, to be utilised in case a Level 3 Amphibian Metamorphosis Assay (AMA, OECD TG 231) indicates thyroid activity of a substance and thus, the need for the generation of additional data. However, the LAGDA is not limited to generating endpoints related to the thyroid modality, as the second half of the study is focused on the estrogen, androgen and steroidogenic (EAS) modalities. Considering that the triggering of a LAGDA is based on concerns around the thyroid axis, questions have been raised as to the value of this additional 10-week period in the study for identifying any additional effects related to (anti)thyroidal activity of substances. Initiation at NF stage 8-10 considers embryo-larval and pre-metamorphic development, already providing a means for assessing potential alternative modes of action and discriminating non-endocrine from endocrine toxicity. Additionally, for the purpose of an assessment on the thyroid modality, generating unrelated data causes needless animal welfare concerns. In an effort to focus on the core strength of LAGDA, we have investigated a modified version of the study, named the ELSATA (Early Life Stage Amphibian Thyroid Assay), which only considers endpoints related to the thyroid modality. In doing so, animal welfare is emphasized, as fewer individuals are used for a shorter total duration, and without decreasing the amount of thyroid-specific data generated. Two thyroid-active

substances, benzophenone-2 (BP-2) and perchlorate (study ongoing) are used to evaluate the practicability of the ELSATA model.

### 1.10.P Legacy and Emerging Contaminants in Wildlife: Recent Advancements in Ecotoxicology and Risk Assessment

#### 1.10.P-We036 Pipping, Hatching, Sex Ratio and Gene Expression Changes in Ducklings Exposed In Ovo to Emerging Per-/Poly-Fluoroalkyl Substances

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Effects of per- and polyfluoroalkyl substances (PFASs) have been observed in wildlife for various endpoints such as oxidative stress, neurotoxicity, immunotoxicity, endocrine disruption, reproduction, and biomass reduction. Although many studies show that environmental PFASs concentrations adversely affect the health of wildlife species, the establishment of a clear link between PFASs exposure and its toxicity is often hampered by unknown effects of additional factors such as environmental conditions, occurrence of co-contaminants, and age and sex of the investigated species. Therefore, exposure experiments under controlled laboratory conditions are needed to plausibly link PFASs exposure to effects in wildlife.

Here, we studied the effects of two unregulated PFASs compounds; perfluoro-4-ethylcyclohexane sulfonic acid (PFECHS) and perfluorododecane sulfonic acid (PFDoDS), alongside the regulated perfluoro octane sulfonic acid (PFOS). Both compounds have been detected in samples from wild bird species. Furthermore, a sex ratio bias towards males has been found in wild ducks. Various PFASs have been shown to interfere with transcriptional pathways linked to growth, development, and reproduction. MicroRNAs are a class of small noncoding RNAs that are involved in the regulation of gene expression at the posttranscriptional level. For certain microRNAs, expression changes have been found after exposure to PFOS and other pollutants. However, the effects of PFASs exposure on microRNA profiles during ontogeny of birds are not well understood.

The aim of this study was to assess PFECHS and PFDoDS developmental effects alongside PFOS, using the mallard duck (*Anas platyrhynchos*) as a model species. We investigated hatching success, sex ratios, and liver microRNA profiles in ducklings exposed *in ovo* to PFOS, PFECHS and PFDoDS.

We observed a lower hatching success in the PFECHS and PFDoDS groups compared to the control and PFOS groups. In addition, the sex ratio in the control group is skewed towards females, while the PFOS group showed a bias towards males. PFECHS and PFDoDS groups have a more balanced sex ratio. Gene expression data will be obtained early in 2024 and will be presented at the conference.

The results of this experiment will not only increase our understanding of the toxicological mechanisms of PFECHS and PFDoDS at a gene expression level during ontogeny of birds, but also will be meaningful for environmental risk assessment and management.

#### 1.10.P-We037 PERSISTENT ORGANIC POLLUTANTS AND THE ROLE OF ANTHROPIC SOURCES IN THE DIET OF AVIAN SCAVENGERS

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Vultures, face declines due to various factors, including anthropization and changes in farming systems. However, there is limited research on the detailed relationship between contaminant levels and individual foraging behavior, especially in scavenger birds exploiting "risky" trophic sources like landfills. This research aimed to assess POPs such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), on Egyptian and griffon vultures, examining their association with the scavenger's diet and trophic strategies using stable isotopes.

In 2021, blood samples were collected from fledging Egyptian (EV, n=14) and griffon (GV, n=15) vultures inhabiting a Natural Park in Navarre, northern Spain, within an area heavily humanized, with shifts in agriculture and livestock practices. Blood's plasma was analyzed for their POP content. Additionally, mantle feathers and hair samples, from vultures' prey, were gathered for stable isotope analyses ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ).

Low concentrations of pollutants were found in both vulture species, with median values within the sub-ppb range. This stands in clear contrast to concentrations reported in other regions worldwide, and below the levels previously recorded for these species in the same study area in earlier decades. Specifically, PCBs were higher in EVs compared to GVs, with medians of 0.564 pg/ $\mu\text{L}$  and 0.282 pg/ $\mu\text{L}$ , respectively. Conversely, OCPs were similar in EVs (median: 0.349 pg/ $\mu\text{L}$ ) and GVs (median:

0.350 pg/ $\mu$ L), although broader pollutant ranges in GV's were observed due to two specimens with exceptionally high concentrations. Dietary models based on isotopic signatures indicated that both species were globally relying on the same food resources. However, a diet more based on carcasses of intensive farming and landfills was associated with higher PCBs, as it was found for Egyptian vultures.

In conclusion, low PCB and OCP concentrations in northern Spain's avian scavengers suggest effective implementation of restrictive regulations, minimizing expected negative effects on fitness. Yet, surveillance is advised, particularly for Griffon vultures exhibiting some high OCP levels, highlighting potential individual exposure variation. This study also underscores stable isotopes' utility in gauging avian scavengers' exposure to contamination sources, especially PCBs, which is crucial in a changing food source landscape with declining extensive livestock and increasing dependence on landfills.

#### **1.10.P-We038 Putting Individual Effects into the Context of Natural Variability. Using Dynamic Energy Budget (DEB) Modelling in Amphibians to assess the Normal Operating Range for Metamorphosis**

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Amphibian assays are important tools in the assessment of thyroid activity of plant protection products. While for human health adversity is considered on the individual level, for non-target species the population is the protection goal. However, it is still unclear how adversity on the population level should be assessed.

EFSA suggested the concept of assessing effects on a population level relative to the normal operating range (NOR) in a report from 2021 on the natural variability of honeybee hives and considered similar approaches also for several mammal species (e.g. common vole, wood mouse) to derive specific protection goals. However, for processes which are plastic even on the organism level, like amphibian metamorphosis, we believe a first step is to assess an individual level NOR. We consider if effects in laboratory studies are within this NOR of individuals, they are not relevant on the individual level and, following this logic, by definition not relevant on the population level.

To assess the NOR of amphibian metamorphosis, a Dynamic Energy Budget (DEB) model was developed which explicitly includes the plasticity of metamorphosis. In an extensive literature review, the most relevant environmental conditions determining metamorphosis rate, and so the natural variability, were assessed. The most relevant factors were included in the DEB model. Additionally, the model has been expanded to reproduce the restructuring of biomass during the metamorphosis climax. In this way we can explicitly simulate the natural variability of the metamorphosis rate on an individual level and potential impacts of plant protection products can be assessed for their relevance in this context. If effects are borderline in the context of natural variability on an organism level, the developed model can also be included in a population model to assess the relevance of these individual effects on a population level.

Since this approach combines models and concepts which were accepted by EFSA for addressing similar issues in other taxa, we believe that this approach can be a way forward in determining the adversity of endocrine-mediated effects on a population level.

#### **1.10.P-We039 Chronic exposure of amphibian aquatic stages to pesticides: a case study with flupyradifurone**

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Amphibians are not routinely considered as part of environmental risk assessment of pesticides, which raises uncertainty about the efficacy of this regulatory process to protect them. As vertebrates, alternative approaches to animal testing like extrapolation from surrogate taxa and effect modelling from pesticide impacts, bring increasing interest. While acute toxicity of chemicals to aquatic stages can be estimated from fish-generated data [2,3], there is little support about the validity of surrogates to cover long-term toxicity. Amphibian development has unique features, including a period of complete starvation during metamorphosis that challenges the development of energy-based effect models. We monitored survival and growth, as a proxy of energetic balance, during a long-term exposure (up to 76 days) of larval green frogs to 0, 0.01, 0.1, 1, 10 or 100 mg/l of the insecticide flupyradifurone applied as Sivanto Prime<sup>®</sup>. The exposure cause a dose-dependent reduction of larval survival during the initial stages ( $p=0.006$ ), with mortality rates of up to 60% at the highest insecticide level. During the metamorphosis transition, percentage of successful individuals was also reduced at the highest flupyradifurone level, although in a non-significant manner ( $p=0.425$ ), which reflects that the stress during larval stage could make that only the strongest individuals reach metamorphosis. Larval growth did not significantly differ among treatments, but a clear trend for arresting growth towards the end of the larval period was seen at concentrations  $\geq 10$  ppm. These results suggest that the period after metamorphosis climax (no energy uptake) is less sensitive to the toxic action than the pre-climax period (positive energetic balance). This pattern should be considered in energy-based models like DEB in amphibians, and also in population models because completing metamorphosis in optimal conditions is essential for juvenile survival and in turn for population dynamics. Funded by EFSA through the project AMPHIDEB (contract OC/EFSA/SCER/2021/12).

### **1.10.P-We040 Amphibians Provide Unique and Sensitive Models in Ecotoxicology: Arguing for Advanced Propagation and Integration in Risk Assessment**

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The unique vertebrate class *Amphibia* is covering the transition to partial terrestrial life style and consists of the three orders *Anura* (frogs), *Urodela* (salamanders), and *Gymnophiona* (caecilians). They developed manifold life styles to inhabit nearly all ecosystems and are very sensitive to environmental factors including chemical substances. Although information about ecotoxicology are available for all three orders, ecotoxicological research has mostly been performed in frogs representing the classical models for endocrinology and developmental biology. In addition, for practical reason research is focusing on lab models such as *Xenopus* but also wildlife species with seasonality should be at least checked for relevancy of lab based results. Classical ecotoxicological tests investigating acute toxicity by lethality without assessing modes of action (MOA) have past and are rarely done for instance the FETAX (Frog Embryo Teratogenesis Assay *Xenopus*) because they are taken over by fish egg tests.

However, a great revival for amphibians as model organisms in ecotoxicology came along with the emerging issue of endocrine disruption (ED) because the endocrine systems of amphibians and other vertebrate classes including humans are quite similar. Starting with endocrine active compounds affecting reproductive physiology of amphibians quite sensitively during larval exposure the establishment of the amphibian metamorphosis assay (AMA) is the most sensitive standardized test guideline (OECD 231) for determining thyroid system disrupting effects. Further test guidelines to assess ED are XETA (*Xenopus* Eleutheroembryonic Assay; OECD 248) and LAGDA (Larval Amphibian Growth and Development Assay; OECD 241), the latter one determining impacts on both, reproduction as well as thyroid system. Recent advancements direct towards an extended AMA that could assess all developmental impacts on reproductive physiology and thyroid system more efficiently and sensitively than any other test. In addition, integrating new approach methods allow to increase the sensitivity as well as the determination of MOA being exemplified by two AMA exposures with the weak antithyroidal compounds Carbamazepine and Tetrabromobisphenol A. Furthermore, amphibians provide phantastic models for assessing ED by using non-invasive behavioural parameters.

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### **1.10.P-We041 Anticoagulant Rodenticides in Wildlife: A Risk for Game Meat Consumers?**

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Game animals are at risk of exposure to anticoagulant rodenticides (ARs), either by the direct ingestion of anticoagulant baits or by the consumption of carcasses of animals poisoned by ARs. Granivorous or herbivorous species such as partridges, ducks, pigeons, hares and rabbits can ingest AR-treated grain or baits because they associate these as potential food resources. Omnivorous species such as wild boar can be also exposed through the consumption of AR-poisoned animals. In New Zealand, second-generation ARs (SGARs) have been detected in the liver of several large game species, and in the case of the feal pigs with hepatic concentrations that can affect blood coagulation of the animals. In Spain, first-generation ARs (FGARs) have been detected in small game species during large scale treatments against voles, and SGARs have been detected in 45% and 12% of the liver and muscle samples, respectively, of wild boar hunted in Barcelona province. Although the observed concentrations in liver and muscle were below those that are likely to be lethal or to affect blood clotting in humans after acute exposures, there is still a need of improving the environmental risk assessment of chronic exposures to SGARs in game meat consumers. In some areas in Spain, SGARs are used to control rabbit overabundance, which may increase the risk of exposure to toxic doses in the consumers of these animals. The high capacity of SGARs to bioaccumulate in liver, the extremely high toxicity of SGARs and the high percentage of human population with an antithrombotic treatment must be considered in the refinement of their risk assessment. This study compiles the information about SGAR prevalence and concentrations in edible tissues of game species as a first step for this assessment. The study also identifies current gaps that should be filled to improve the evaluation of the risk on game meat consumers and the recommendations for risk mitigation. This study is developed under the framework of the Cost Action CA22166 - Safety in the Game Meat Chain (SafeGameMeat).

### **1.10.P-We042 Anticoagulant rodenticide exposure of two mammal species, American badger and fisher from British Columbia Canada.**

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There are increasing reports from many jurisdictions of mammalian carnivores with elevated exposure to anticoagulant rodenticides (ARs). A proportion of animals tested have exhibited symptoms of anti-coagulant poisoning. Most of those samples from the literature, but not all, were from locations near to agricultural or urban development. For the present study, we tested 69 samples of two species of mammal, the American badger *Taxidea taxus*, an endangered species in British Columbia, and a furbearer, the fisher *Pekania pennanti*, collected over the period 2004 to 2018 from locations in southern British Columbia, Canada. Of 59 badgers analyzed, 46% (27) contained residues of both second generation (SGAR) and first generation (FGAR) compounds, with 10% (n=6) exposed to only SGARs, and 14% (n=8) exposed to just FGARs. The mean total exposure was 0.20 µg/g while the geometric mean was 0.15 µg/g, (range: 0.004-2.138 µg/g ww). Of the exposed badgers (n=41), 73% (n=30) are exposed to at least two AR compounds, 49% (n=20) are exposed to at least three AR compounds. The



breakdown of exposure by compound was: bromadiolone (n=33), brodifacoum (n=21), chlorophacinone (n=20), diphacinone (n=17), warfarin (n=12), difethialone (n=12). Of the badger liver samples, 17 contained > 0.100 µg/g ww  $\Sigma$  ARs with a range of 2.14 – 0.146 µg/g ww. Six females were lactating all of which were exposed to ARs. Fishers were collected over an earlier timeframe, 2003 – 2009. They were less exposed, with 2 out of 10 individuals testing positive, one of which was exposed to warfarin (0.05 µg/g) and high levels of bromadiolone (0.528 µg/g), and the second individual tested positive for brodifacoum (0.013 µg/g). We discuss likely exposure pathways and toxicological implications of these results for badger conservation and population recovery.

**1.10.P-We043 Anticoagulant Rodenticides in Birds of Prey in Switzerland – Towards an Appraisal of Threshold Values**  
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Due to their widespread use for rodent pest control, the presence of anticoagulant rodenticides (ARs) in the environment is ubiquitous. These substances, especially the second generation ARs, have persistent, bioaccumulative and toxic properties that can lead to secondary poisoning of non-target organisms. Exposure of non-target organisms, such as small predators or birds of prey, to ARs is well documented internationally. However, the situation in Switzerland had so far only been scantily investigated. To fill this gap, a reliable and sensitive analytical method (LC-MS/MS) to quantify ARs in liver samples was established and a first set of wildlife liver samples was analyzed for seven approved substances (brodifacoum, bromadiolone, coumatetralyl, difenacoum, difethialone, flocoumafén, warfarin). Samples included livers of foxes, birds of prey, hedgehogs and fish. In almost all samples analyzed, up to four different ARs were detected with maximum liver concentrations of 1100 ng/g (fox), 450 ng/g (bird of prey), 2 ng/g (hedgehog) and 40 ng/g (fish). A comparison with data collated from literature showed similar exposure in biota from surrounding countries.

To date, there is no broad consensus as to which AR concentrations lead to toxicosis and must therefore be considered problematic. Some experimental toxicity data are available from studies in which animals were fed food or prey laced with AR. However, only a few species have been studied in this way, often with low numbers of animals and only testing single substances and not mixtures. Another approach to derive threshold values involves the combination of measured AR liver burdens and the determination of the cause of death by means of pathological examinations. For this reason, we started a new project where we focus on birds of prey that died during care in bird rehabilitation centers. We aim to extend monitoring of AR exposure in Switzerland and combine it with pathological examinations of birds. The data obtained will then be fed into established models to determine AR threshold values and allow for improved risk assessment concerning non-target organisms. Additional data are also crucial to assess future trends in AR exposure and for evaluating the efficiency of possible future changes to AR application strategies.

**1.10.P-We044 Anticoagulant Rodenticide Contamination and Coagulation Capacity Assessment in Long-Eared Owls (*Asio otus*) from a Mediterranean Agricultural Landscape**

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Anticoagulant rodenticides (ARs) are chemical compounds widely employed for rodent population control in various anthropogenic landscapes. These highly toxic substances, known for their bioaccumulative nature in the tissues of consuming individuals, not only induce mortality in target rodents but also pose a threat to the populations of predators and scavengers that feed on these contaminated prey. Here, the long-eared owl (*Asio otus*) is used as a biomonitoring species to assess the presence and distribution of ARs in different agricultural landscapes within a Mediterranean semiarid region of southeastern Spain. Blood samples were extracted from 69 nestlings belonging to 38 nests in the Region of Murcia and analysed using HPLC/MS/MS. Additionally, plasma samples were obtained to evaluate clotting function through prothrombin time (PT) testing as a biomarker of exposure and effect. Our analysis revealed an almost absolute prevalence (98.6%), with no variation across different agricultural landscapes and multiple ARs detected in most samples (82.6%). The most commonly detected ARs were second-generation compounds (SGARs), particularly flocoumafén (88.4%). Total concentration ( $\Sigma$ ARs) ranged from 0.06 to 34.18 ng mL<sup>-1</sup> and the highest levels were found in a study site dominated by intensive agriculture. However, no correlation was found between  $\Sigma$ ARs and the different land uses calculated within 1 and 1.5-km buffers around each nest, which was established as a proxy of the adults' home range. Furthermore, blood  $\Sigma$ AR levels showed a positive correlation with PT values measured in the corresponding plasma samples ( $Rho=0.547$ ,  $p=0.000$ ), highlighting the presence and quantifiable effects of these compounds even at low blood concentrations. These findings underscore the widespread presence of ARs across the study area, indicating early exposure in long-eared owl nestlings. Our results also suggest that exposure in this owl species is chronic, which could potentially lead to medium or long-term toxic effects due to the progressive accumulation of

ARs in the organism. Moreover, the long-eared owl proved to be an excellent sentinel species for monitoring environmental contamination in agricultural landscapes, suggesting that other wildlife from the same area may also be exposed and affected by the detrimental effects of AR compounds. This study emphasizes the urgent need for effective strategies to mitigate the impact of ARs on ecosystems and protect non-target wildlife.

#### **1.10.P-We045 Environmental Factors Influencing Anticoagulant Rodenticide Exposure in Common Kestrels (*Falco tinnunculus*) and Barn Owls (*Tyto alba*) from Southeastern Spain**

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Anticoagulant rodenticides (ARs) act by disrupting blood clotting and causing fatal haemorrhages in target rodents. Previous studies have demonstrated their widespread occurrence in the environment, moving through the food chain and accumulating in top predators such as birds of prey. These predatory birds also display higher sensitivity to ARs compared to other avian species, raising concerns about their potential impact on raptor populations, particularly for species inhabiting agricultural landscapes near human activities. Here, we aimed to assess the exposure to second-generation ARs (SGARs) in two farmland bird of prey species with different foraging habits. For that purpose, we blood-sampled barn owl (*Tyto alba*,  $n=54$ ) and common kestrel (*Falco tinnunculus*,  $n=70$ ) nestlings in the Region of Murcia (southeastern Spain), during the breeding seasons of 2021 and 2022. Samples analyzed using HPLC/MS/MS revealed high prevalence in both species - 50% in *T. alba* and 68% in *F. tinnunculus* - with the presence of multiple ARs in 16% and 32.9% of the individuals, respectively. Flocoumafen was predominant in kestrels, while bromadiolone in barn owls, with SGAR levels ( $\Sigma$ SGARs) ranging from 0.07 to 11.52 ng mL<sup>-1</sup> in the kestrel and 0.03 to 3.75 ng mL<sup>-1</sup> in the barn owl. Environmental variables that could affect the prevalence (study site, land uses, livestock farms, and human population density) were analysed within a 1-km buffer around each nest. This study revealed that SGAR prevalence in kestrels was related to the extension of artificial surfaces (roads, buildings, industrial areas, parkings, etc.), with no significant differences observed among study sites. In contrast, barn owls showed the higher prevalence in the most densely inhabited study site, with human population density emerging as the most explanatory factor. Hence, it seems that the kestrel - with a more generalist and flexible diet - frequently forages near buildings and urban centers where it would be prone to come into contact with AR contaminated prey. Conversely, the barn owl, although primarily feeding on rodents, often hunts in open spaces away from settlements. Therefore, contamination risk would be greater for owl nests located near urban areas, with higher presumed AR use and presence of target rodents such as rats (*Rattus sp.*). Our findings emphasize the need for responsible application practices and conservation efforts to safeguard raptor populations in Mediterranean agricultural regions.

#### **1.10.P-We046 Impact of Changes in Governance for Anticoagulant Rodenticide Use on Non-target Exposure in Buzzards (*Buteo buteo*)**

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Birds of prey are susceptible to non-target exposure to anticoagulant rodenticides (ARs) used in pest management. An industry-led stewardship scheme introduced new rules on the use and sale of rodenticide products across the UK in 2016, with the aim of reducing this risk. To determine if this intervention was effective, exposure to second generation anticoagulant rodenticides (SGARs) was measured in buzzards. Liver samples from 790 buzzards collected between 2005 and 2022 were analysed and the percentage presence and concentrations of SGARs from pre-stewardship and post-stewardship samples were compared.

There was no statistically significant change in the percentage of buzzards exposed to bromadiolone, difenacoum or summed SGAR residues after the introduction of stewardship. The percentage of buzzards exposed to brodifacoum increased significantly post-stewardship, from 8% to 27%. There were no significant changes in the concentrations of individual SGARs post-stewardship but concentration of summed SGARs increased significantly post-stewardship. Buzzards were significantly more likely to be exposed to multiple SGARs post-stewardship.

These findings echo similar findings in other wildlife monitoring studies and suggest that the industry-led stewardship scheme has not yet had the intended impact of reducing SGAR contamination in non-target wildlife.

#### **1.10.P-We047 Exposure of piscivorous avian predators to second-generation anticoagulant rodenticides**

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Exposure of wildlife to anticoagulant rodenticides (ARs) has been extensively monitored worldwide for a variety of terrestrial species directly or indirectly linked to pest rodents via the terrestrial food web. In recent years, the scientific focus of environmental AR monitoring extended to AR emissions to the aquatic environment, demonstrating the relevance of aquatic

exposure pathways such as sewer baiting and bait application in close proximity of surface water bodies. Hence, in regions with pronounced rodent control measures in Germany, biocidal ARs were frequently detected in Eurasian otters (*Lutra lutra*), a primarily piscivorous mammalian predator, in spite of strict regulations regarding the sale, supply, and use of ARs. Here, we present additional evidence that second-generation ARs entering the aquatic environment and accumulating in native freshwater fish, are transferred along the aquatic food chain, possibly posing a threat to piscivorous predators. Liver samples of 96 cormorants (*Phalacrocorax carbo sinensis*), an exclusively piscivorous, migratory avian predator, were analyzed regarding residues of all eight active ingredients used in biocidal ARs in Germany. All of the randomly investigated cormorants from different German regions (Bavaria, Rhineland-Palatinate, Saxony) had been shot for nature conservation reasons based on state-specific species protection exception regulations between 2020 – 2023 (outside breeding season). In summary, hepatic residues of biocidal ARs were detected in 46% of investigated cormorants (max. individual total hepatic AR concentration of 35 ng/g based on liver wet weight). Second-generation AR active ingredients brodifacoum, difenacoum, and bromadiolone were detected almost exclusively, reflecting their estimated market share in Germany. Without doubt, improvements of regulatory measures will be required to mitigate the almost ubiquitous occurrence of hepatic second-generation AR residues in non-target wildlife as the potential ecotoxicological consequences thereof are yet unknown.

#### **1.10.P-We048 Biomonitoring of metals and second-generation anticoagulant rodenticides at the pan-European scale from 1996 to 2021 with wild Common Buzzards (*Buteo buteo*)**

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Avian predators have been widely used for biomonitoring due to their long lifetime and large foraging areas. However, many data from top-predator monitoring are temporally or regionally fragmented, which complicates long-term and large-scale chemical risk and hazard assessments. To fill this knowledge gap in the assessment of chemicals in the terrestrial compartment at a large scale, we conducted a pan-European scale biomonitoring using Common Buzzards (*Buteo buteo*) as sentinel species. We present here our initial results on the temporal trend of two types of chemical contaminants posing threats to terrestrial wildlife, heavy metals and second-generation anticoagulant rodenticides (SGARs).

We collected buzzards found dead or dying in the wild from 11 European countries from 1996 to 2021. Livers from several individuals within a region of a country were pooled to increase sample mass for multiple-contaminant analysis. Concentrations of three metals (mercury, cadmium and lead) and five SGARs (bromadiolone, difenacoum, brodifacoum, flocoumafen and difethialone) were measured in 64 pooled samples and compared between countries. The spatiotemporal trend of each contaminant and the number of samples exceeding a threshold referred from the literature were modelled.

Except for cadmium, which was significantly lower in Spain than in Germany, no significant difference between countries was observed in metal and SGAR concentrations. There was no significant temporal trend in metal concentrations, while summed SGAR concentrations ( $\Sigma$ SGARs) decreased from 1997 to the end of the 2000s and then increased. Regarding each SGAR, only brodifacoum showed a similar temporal trend as  $\Sigma$ SGARs, but no other SGAR significantly varied over time. Meanwhile, the proportion of samples exceeding the threshold of at least one metal or SGAR significantly differed between before and after 2015. For each contaminant, the same result was observed only in brodifacoum.

Overall, metals in buzzards showed a spatial variation but did not change over time. In contrast, SGARs did not spatially vary but changed over time. Potential health risks from the analysed chemical contaminants on buzzards increased around the middle of the 2010s, mainly due to high brodifacoum concentrations. Interpretation of these findings needs to consider the

chemical regulation changes over time in Europe, the potential disparity in the regulatory authorities, and the variety in the ecology of the bird between countries.

#### **1.10.P-We049 Lead Exposure of Scavenging Birds Due to Accidental Ingestion of Lead Ammunition: Geographic and Species Variation in Canada**

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Multiple bird species, including raptors and corvids, accidentally ingest spent lead ammunition while scavenging on gut piles of hunted game, which can result in acute and chronic toxicological effects. This phenomenon has been reported worldwide and for some bird species is associated with population-level impacts. To evaluate lead poisoning of scavenging birds across Canada, we initiated a national scale study in 2018, with the objective of using a pan-Canadian database to (i) assess the extent of lead exposure of scavenging birds and (ii) identify bird species and regions at higher risk. Through a collaboration between Environment and Climate Change Canada, the Canadian Wildlife Health Cooperative, territorial governments, and other partners, a sample set of close to 1000 livers has been obtained from dead and moribund birds brought to wildlife organizations. Currently, bald eagle is the most sampled species (n = 423) followed by red-tailed hawk (n = 139), common raven (n = 101), great horned owl (n = 90), golden eagle (n = 50), merlin (n = 38), American crow (n = 35), and turkey vulture (28). Measurement of lead burdens in the livers is on-going, though preliminary concentration data (n = 792) were compared to an established threshold of 20 mg/g dry weight (dw) associated with a high risk of clinical lead poisoning. Bald eagle, golden eagle, and turkey vulture showed the greatest lead exposures, with 12-15 % of birds having liver concentrations in exceedance of 20 mg/g dw. A few individuals of red-tailed hawk and great horned owl also showed exceedances of that threshold, though at a lower frequency of 1-2 %. None of the corvid species had liver concentrations of lead above the threshold (maximum concentrations  $\leq$  5 mg/g dw). A comparison of bald eagle lead concentrations among regions of Canada showed considerable geographic variation in exposure, with threshold exceedances ranging from 4-23%. Antimony concentrations of bird livers were also examined because antimony is used as a hardener in lead ammunition alloys. A positive correlation was found between bird liver concentrations of antimony and lead, which is consistent with lead ammunition being a dominant source of lead exposure to the birds. This national-scale Canadian dataset will provide a baseline to track long-term trends in lead exposure of a representative suite of raptors and corvids.

#### **1.10.P-We050 Feathers to the Rescue: A Non-Destructive Tool for the Detection of Metals and Metalloids**

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Heavy metals and other inorganic elements are widely distributed in the environment through the geological cycle. Additionally, they are released due to various human activities such as industrialization, combustion, smelting processes, vehicle emissions, mining, agricultural runoff, and oil activities, among others. Some inorganic elements, such as Zn, Cu, and Se, are essential for organisms, playing a crucial role in their biological functions. In contrast, elements like Cd, Hg, and Pb are non-essential and have been shown to have adverse effects on birds. These inorganic elements occur naturally in the environment, implying a constant level of exposure. Birds have developed adaptations to cope with this exposure, and concentrations of essential elements typically remain in balance in birds unless the exposure is significantly high and disrupts normal homeostatic processes. The aim of this study was to assess the presence of inorganic elements in the feathers of green-winged teals and snow geese wintering in Laguna de Santiaguillo, Durango, Mexico, and also to evaluate the usefulness of feathers as indicators of exposure to these contaminants.

During the hunting season 2021-2022, 27 specimens of snow geese (*Anser caerulescens*) and 30 green-winged teals (*Anas crecca*) were collected. Feathers were taken from the collected birds, and the determination of metals was carried out using the voltammetry technique.

The concentrations of inorganic elements showed the following pattern: In *A. crecca*: As > Cr > Cu > Ni > Hg > Zn > Sn > Pb > Al > Cd, while in *A. caerulescens*: Ni > Cr > Cu > As > Hg > Al > Sn > Zn > Pb > Cd. For both species, Cr and Cu were the elements observed with higher concentrations, and Cd was the lowest concentration; the rest of the elements were distributed differently between the species. Although the average concentrations of inorganic elements in both species do not suggest significant exposure, some individuals with particularly high concentrations of Pb and Hg were observed.

The use of feathers as biomarkers of exposure to metals and metalloids is highly beneficial since when these elements circulate in the blood, they adhere to the proteins present in the feathers. Thus, they accumulate throughout the development and growth process of feathers. Once feather growth ceases, it seals, ceasing to interact with the bird's physiology, reflecting the levels of metals accumulated during its growth or molt period.

### **1.10.P-We051 The Red-billed Chough (*Pyrrhocorax pyrrhocorax*) as a Sentinel Species for the Input of Heavy Metals from the Tajogaite Volcano into the Environment of La Palma, Canary Islands**

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Geothermal activities, such as volcanic eruptions, represent one of the most important natural sources of metal emissions. These events can have an impact on the health of ecosystems incorporating large amounts of different pollutants.

On September 19th of 2021, in Cumbre Vieja (La Palma, Canary Islands), began the most recent volcanic eruption in Spain (Tajogaite volcano), which ended on December 25th of the same year. Being such a recent event, the effects of ash exposure on wildlife and humans have been scarcely studied.

The red-billed chough (*Pyrrhocorax pyrrhocorax*) is considered a symbol or "flagship species" of the island's natural values. Knowledge of the impact that the Tajogaite volcano may have had on its population is essential to assess the present and future conservation status of this species.

The main objective of this study is to evaluate the exposure to levels of contamination by heavy metals in wildlife in the area after the eruption.

To this end, the red-billed chough (*Pyrrhocorax pyrrhocorax*), named locally as "graja" has been considered as a monitoring species by using moulted wing feathers as monitoring tool of heavy metals exposure. Feathers collected before and after the eruption were used to compare both pollution scenarios.

Twenty-nine red-billed chough feathers were sampled (15 before and 14 after the eruption), on which the following metals were analyzed by IPC-MS: Al, Sr, Fe, Cu, Zn, Co, Ni, Cr, As, Cd, Pb, V, Se, Hg. Samples of ashes from the volcano eruption were also analyzed.

Most of the metals except of Cu, Zn, As and Pb, showed significant lower concentrations before than after the eruption, suggesting a common origin for most of the metals studied, which could be directly related to the recent eruption. Moreover, the metal and mineral profiles in feathers and volcanic ashes were very similar.

As a complement, a study of the levels of the same metals in internal organs of chough carcasses found dead before and after the eruption is being carried out in order to obtain more information on the possible consequences not only for the health of this species, but also for other species, including humans.

### **1.10.P-We052 Why are flamingo eggs not good indicators of environmental pollution?**

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We present the first report on metallic element concentrations in egg contents of any flamingo species worldwide. Lesser Flamingo Eggs *Phoeniconaias minor* were sampled at Kamfers Dam, Kimberley, South Africa. As one of the longest-lived bird groups (up to 50 years), we expected birds of the Family Phoenicopteridae to accumulate metals from the highly saline waters where they feed and transfer them to their eggs. However, the concentrations of metals were lower than expected when compared with shorter-lived large aquatic birds from the same region, possibly indicating maternal physiological processes that protects the embryo. Large inter-egg variations reflect their wide-ranging nomadic movements. Strontium concentrations in eggshells exceeded toxic reference values, and copper concentrations in egg contents may cause reproductive stress.

Although there was no molar association between selenium and mercury, selenium was in excess. We identified various mechanisms by which flamingos have evolved to potentially remove metals from their bodies, such as excretion through feathers, salt glands, uropygial glands, and crop milk. In many respects, they could be considered marine birds. While embryonic flamingo chicks may be protected *in ovo*, we identified post-hatching routes of uptake by the chicks not previously recognised. Chicks consume their eggshells to an amazing extent, and the parents feed them crop milk akin to breast milk in mammals. We consider neither *P. minor* egg contents nor eggshells as good indicators of environmental metallic pollution. Our findings suggest a more nuanced approach to monitoring and protecting Phoenicopteridae from pollution.

### **1.10.P-We053 Compound-specific Stable Isotope Analyses of Amino Acids Reveal Drivers of Mercury Concentrations in Steller Sea Lions and their Prey**

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Total mercury concentrations ([THg]) exceed thresholds of concern in some Steller sea lion (*Eumetopias jubatus*) tissues from certain portions of the Aleutian Islands, Alaska (USA). We applied compound-specific stable isotope analyses (CSIA-AA) of both carbon and nitrogen in amino acids from fish muscle tissue to quantify the proportional contributions of primary production sources and trophic positions of eight prey species ( $n = 474$  total) that are part of Steller sea lion diets. THg analyses of fish muscle, coupled with monomethylmercury analyses of a subset of samples, substantiated previous findings that fishes from the west of Amchitka Pass, a discrete oceanographic boundary of the Aleutian Archipelago, have higher muscle Hg concentrations relative to fishes from the east. The  $\delta^{13}\text{C}$  values of essential amino acids (EAAs) in fish muscle demonstrated that although most fishes obtained their EAAs primarily from algae, some species varied in the extent to which

they relied on this EAA source. The  $\delta^{15}\text{N}$  values of phenylalanine (0.9 to 7.8 ‰), an indicator of the isotopic baseline of a food web, varied widely within and among fish species. Trophic position estimates, accounting for this baseline variation, were higher from the west relative to the east of the pass for some fish species. Trophic magnification slopes using baseline-corrected trophic position estimates indicated similar rates of Hg biomagnification to the east and west of Amchitka Pass. Multiple linear regression models revealed that trophic position was the most important driver of fish muscle [THg] with less variation explained by other parameters. Thus, higher trophic positions but not the rate of Hg biomagnification to the west of Amchitka Pass may play a role in the regional differences in both fish and Steller sea lion [THg]. Although, differences in Hg contamination and uptake at the base of the east and west food webs could not be excluded. To better identify the drivers of observed temporal patterns in Steller sea lion [THg], we also performed CSIA-AA of nitrogen on Steller sea lion whiskers. Results indicate cyclical variation in the isotopic baseline along whiskers (i.e., over time), suggesting Steller sea lions are foraging and obtaining Hg from different locations seasonally. The application of CSIA-AA to Steller sea lions and their prey is allowing for a more nuanced perspective of Hg pathways in this remote region of Alaska.

#### **1.10.P-We054 Dietary shift in Southeast Alaskan wolf populations leads to increased mercury exposure**

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Although all wolves (*Canis lupus*) express a high degree of dietary plasticity, the coastal wolves in Southeast Alaska, USA (*Canis lupus ligoni*) have demonstrated an extremely diverse diet dominated by ungulates but including marine resources opportunistically throughout their range. Recent studies have shown a drastic dietary shift towards sea otters (*Enhydra lutris*) in the region near Glacier Bay National Park and Preserve (GLBA), specifically for the wolves inhabiting Pleasant Island and the Gustavus Forelands. Sea otter populations were decimated by the fur trade prior to their protection in 1911. Following their reintroduction to Southeast Alaska in the 1960s, sea otters have since increased in numbers, especially near GLBA. This recovery has allowed the ranges of sea otters and wolves to overlap, resulting in a shift in wolf diet from terrestrial herbivores (ungulates such as deer and moose) to a marine predator (sea otters). As monomethylmercury is produced and biomagnifies mainly in aquatic food webs, we expected to find higher concentrations of total mercury in wolves with strong connectivity to marine food webs. To determine baseline contamination and identify the trophic transfer of mercury, we measured total mercury concentrations and the stable carbon and nitrogen isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values) in fur from wolves and sea otters, as well as in the tissues of intertidal fishes and invertebrates from Pleasant Island and the Gustavus forelands. We also compared stable isotope and total mercury concentrations of Pleasant Island/Gustavus wolves with data from wolves (n=118) across Southeast Alaska. Our results indicate substantial variation in fur total mercury concentrations (0.05 to 17.1 ppm) and  $\delta^{13}\text{C}$  (-26.4 to -14.6‰) and  $\delta^{15}\text{N}$  (4.5 to 14.0‰) values. Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were tightly correlated with fur total Hg concentrations demonstrating that dietary reliance on marine resources is a major driver of Hg exposure for wolves across Southeast Alaska. Due to their predation of sea otters, wolves from Pleasant Island had the highest total Hg concentrations, which may ultimately affect their health.

#### **1.10.P-We055 Uncovering Effects with Molecular Techniques and Primer Design: Chronic Mercury Exposure and gene expression in Svalbard Reindeer**

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Despite its remote Arctic location, Svalbard has significant levels of Mercury contamination, (Hg). Hg travels to the Arctic via long-range atmospheric transport, from industrialised areas all over the world. As a result, the Arctic cryosphere is the second-largest sink of legacy mercury on Earth. As temperatures rise in the Arctic, historically stored mercury may remobilise to other environmental compartments. The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) is the largest herbivore of the archipelago, and an essential part of the terrestrial ecosystem. They consume vascular vegetation, moss, and lichen, which take up Hg from air and water. Top predators include humans, polar bears, and Arctic foxes.

This project investigates low-level, chronic effects in Svalbard reindeer following a life-long exposure to environmental concentrations of mercury. This is done by assessing the regulation of genes associated with oxidative stress and mercury detoxification in liver and muscle samples from Svalbard reindeer. Genes of interest include metallothionein, selenoprotein, glutathione reductase and hepcidin. The project includes primer design development and thus the creation of new primers that can be used for further research. The results from the genetic expression will be compared to the mercury concentration in these tissue samples.

To extend the project and support assumptive expression results, bioassays and blood smear analyses will also be included.

#### **1.10.P-We056 The Chemical Load of Pilot Whales in Arctic Waters**

*Halla Weihe Reinert and Katrin Hoydal, Environmental Protection, Faroese Environment Agency, Faroe Islands*

Long-finned pilot whales in the North Atlantic Ocean are burdened by vast quantities of chemicals transported by air and water from distant places. Due to its position in the food web, these chemicals are bioaccumulated to a high degree. The pilot whale has been a staple diet of the Faroese people for centuries, yet concerns have been raised about its chemical profile for decades.

The Faroese participation in the Arctic Monitoring and Assessment Programme (AMAP) began in 1997, focusing on the monitoring of heavy metals and persistent organic pollutants (POPs) in selected animals, including the pilot whale, along with supporting parameters. One of the main objectives is the generation of temporal trend analyses of contaminants in different tissues in different animals as indicators of environmental pollution. Retrospective analyses have also been done to complement already existing data.

Temporal trends were analysed using a robust regression approach to detect trends as well as an ordinary log-linear regression. The analyses were successfully applied to individual time-series for 59 compounds in four different tissues, resulting in 73 time-series. Of these, 29% show an increasing trend, 36% show a decreasing trend, and 18% show no detectable trend. However, only 34 time-series have a statistical power over 80%, which indicates the need for continued monitoring.

#### **1.10.P-We057 Ultraviolet Absorbents and Industrial Antioxidants in Tissues of the Canadian Arctic Wildlife**

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Ultraviolet (UV) absorbents and industrial antioxidants are plastics associated contaminants and they have been detected in the environment and are known to cause different types of toxicities in exposed organisms. However, the distribution of these contaminants in Arctic wildlife remains poorly understood, hindering comprehensive risk assessment and policy development. This study analyzed benzotriazole UV stabilizers (BZT-UVs), UV filters (UVFs), synthetic phenolic antioxidants (SPAs), and secondary amine antioxidants (Ar-SAs) in the liver, muscle, or blubber of seabirds, marine mammals, and fish from the Canadian Arctic ( $n=278$  samples) collected between 2017 and 2021. Contaminants were analyzed via gas chromatography-mass spectrometry. Seabirds in eastern Nunavut showed elevated levels of BZT-UVs, UVFs, Ar-SAs, and 26DTBP (2,6-di-*tert*-butylphenol) in the liver compared to other species. UV328 (2-(2H-benzotriazol-2-yl)-4,6-di-*tert*-pentylphenol), recently added to Annex A of the Stockholm Convention, exhibited higher accumulation in thick-billed murre livers (mean concentration  $45\pm 18$  ng/g wet weight;  $n=28$ ) than reported in seabirds' liver or egg from other Arctic regions. This suggests higher potential exposure of murre in this colony to UV328, warranting further investigation. The southern Hudson Bay polar bear population was more contaminated with 26DTBP, UV329 (2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethyl butyl) phenol), and 4MBC (4-methylbenzylidene camphor) compared to the western population. Liver UV329 levels were approximately twice as high as the levels in Svalbard polar bears' blood. Eastern Beaufort beluga and sea-run Arctic char from Northwest Territories and Nunavut, respectively, had relatively the lowest contamination levels of the target contaminants. Regarding mammal tissue distribution, walrus and ringed seals showed higher  $\sum$ BZT-UVs in blubber than muscle or liver, with no trend for  $\sum$ UVFs and  $\sum$ Ar-SAs. On the contrary, ringed seals from Northwest Territories displayed higher levels of  $\sum$ SPAs in the liver than the blubber. Interestingly, no correlation was found between the microplastic accumulation reported in the gastrointestinal tract and target contaminant concentrations in these tested species. These findings establish a baseline for monitoring UV absorbents and industrial antioxidants in the Arctic, which is crucial for comprehensively understanding the plastic pollution in the Arctic food web.

#### **1.10.P-We058 Time trends (1996-2021) in PCB and PBDE congeners and in $\Sigma$ PCBs and $\Sigma$ PBDEs residue concentrations in the common buzzard *Buteo buteo* in 11 European countries in relation to restrictions on chemicals use**

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This study aims to explore the utility of long-term contaminant monitoring in raptors as sentinels to assess the effectiveness of chemicals restrictions in Europe. EU regulations, notably Directive 96/59/EC on PCBs, Directive 2003/11/EC on certain PBDEs and the REACH Regulation EC 1907/2006, together with the Stockholm Convention (2001), have been introduced to reduce the threats posed by persistent, bioaccumulative and toxic substances to human and wildlife health, although their transposition or ratification and implementation has varied by country.

Top predators (also called apex species) such as raptors are frequently used as sentinels for bioaccumulating chemicals, with potential for regulatory applications including early warning of emerging contaminants, chemicals risk assessment and assessment of efficacy of chemical risk management measures. Such applications offer substantial promise in relation to EU ambition to limit harm from chemicals to both wildlife and human health. However, little research has explored the potential for such applications at Europe-wide scale, in particular assessment of efficacy of risk management measures.

This study used the common buzzard *Buteo buteo*, an apex species, which is widely distributed in Europe and has been suggested as a suitable species for systematic monitoring of contaminant trends in terrestrial food webs at large spatial scales. We analysed for 31 PCBs and 23 PBDEs in 64 buzzard livers from 11 European countries (Austria, Belgium, Finland, Germany, Greece, Italy, Portugal, Romania, Slovenia, Spain, Switzerland) for the period 1996-2021 using GC-MS. We explored time trends for the number of PCB and PBDE congeners and for residue concentrations. We also explored differences in time trends between substances and countries. In particular, we assessed possible explanations for any observed reductions in the numbers and/or residue concentrations of PCBs and PBDEs over time, at pan-European and at national scales, subsequent to restrictions on these substances. We discuss implications for the further application of monitoring of chemicals in terrestrial apex species to assess the effectiveness of regulatory restrictions at pan-European and national scales.

#### **1.10.P-We059 Title: From soils to apex species: chemical pathways, effects and impacts on terrestrial biodiversity and ecosystem services and applications for better chemicals management**

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TerraChem (*From soils to apex species: chemical pathways, effects and impacts on terrestrial biodiversity and ecosystem services and applications for better chemicals management*) is a three-year collaborative project running from November 2023.

The European Commission's *Chemicals Strategy for Sustainability* aims to minimise the risk of chemicals to wildlife (and human) health. However, the number of chemicals in use far exceeds capacities of conventional risk assessment, registration data is often inadequate and risk assessment does not consider damages to biodiversity and ecosystem services. Real-world contaminant monitoring and in silico modelling can help to assess exposure and effects and damages on biodiversity and ecosystem services, and are increasingly recognised as important in the prioritisation of substances for environmental risk assessment. However, their application in the terrestrial environment has to date been limited.

TerraChem addresses this gap by aiming to develop, demonstrate and apply a novel systems approach, integrating monitoring, environmental modelling, data management, analytical tools and user guidance to better understand exposure of terrestrial abiotic media (soil, soil pore water) and biota across trophic levels (plants to primary and secondary consumers to apex species) to the universe of environmentally-relevant anthropogenic chemicals and resulting damage to biodiversity and ecosystem services. TerraChem will enable more efficient environmental risk assessment of chemicals in the terrestrial compartment and more effective prevention and mitigation, accelerating achievement of the EU's zero pollution ambition.

TerraChem's objectives include: (1) understanding routes of exposure to chemicals in wildlife, including routes and extent of trophic transfer, for selected food chains in representative terrestrial biomes across Europe; (2) modelling source-to-receptor



pathways of selected chemical contaminants for terrestrial ecosystems, and link organism and species effects to damage on genetic and functional diversity and on relevant ecosystem services; (3) developing tools and guidance for regulatory and practice uptake of TerraChem output to optimise current environmental risk assessment of chemicals and improve risk management measures, thereby reducing damage to biodiversity; and (4) developing a TerraChem Data Management System and Dashboard as a One-Stop Shop for data on contaminants in terrestrial biodiversity in Europe.

#### **1.10.P-We060 The TerraChem approach to monitoring chemicals exposure and mixture effects in real-world terrestrial food chains**

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Knowledge on exposure to and effects of chemicals in terrestrial ecosystems in Europe is limited. TerraChem is a collaborative 3-year project running from November 2023. The overall objective of Work Package 1 (WP1) is to understand routes of exposure to chemicals in wildlife, including extent of trophic transfer, for selected food chains (soil and soil water – plants – primary and secondary consumers – apex species) in representative terrestrial biomes. WP1 specific objectives are: (1) to detect and determine chemicals and predominant chemical mixtures present; (2) to understand routes of exposure, including extent of trophic transfer and magnification; (3) to explore patterns for individual contaminants and predominant mixtures in terrestrial trophic chains; (4) to elucidate toxic effects of chemical mixtures using an effects-based approach; and (5) to identify and collate secondary data on contaminants along trophic chains in terrestrial ecosystems in Europe.

WP1 involves six one-country cases in Europe, across representative biomes (e.g. farmland, woodland, grassland) and food chains (e.g. carnivorous mammal, insectivorous mammal), plus one pan-European case study (barn owl *Tyto alba* food chain, six countries). Food chain samples will be analysed by wide-scope target analysis (>2400 substances) and suspect and non-target screening (>95000 substances) using LC- and GC-HRMS, covering the more-or-less full universe of environmentally-relevant anthropogenic chemicals, their transformation products and metabolites. We will explore patterns of individual contaminants and of contaminant mixtures. Routes of exposure and trophic transfer and magnification will be further elucidated using compound-specific amino acid isotope analysis. Mixture effects, at the level of the individual organism, will be explored using a battery of bioassays.

WP1 primary data (and secondary data on contaminants in terrestrial food chains, extracted from previous landscape-scale studies in Europe) will be used in WP2 to train models on source-to-damage chemical pathways in terrestrial ecosystems. WP1 and WP2 outcomes will be used to improve current risk assessment practice and measures (WP3) to better protect biodiversity in Europe. WP1 data will be made available through a new TerraChem database for contaminant data in terrestrial biota in Europe, hosted by NORMAN.

#### **1.10.P-We061 Unveiling the chemical fingerprint of xenobiotics in different otter tissues through HRMS-based targeted and untargeted workflows**

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Anthropogenic activities are one of the main contributors to the introduction of chemicals in the environment and research findings indicate a constantly increasing number of xenobiotics, which are distributed in various environmental compartments. Through their systematic monitoring, insights on their potential harmful properties can be revealed, making their prioritization for the addition in the future legislation more efficient. Apex predators, such as Eurasian otters (*Lutra lutra*), are commonly used as sentinels to assess the effectiveness of chemical regulations, since they possess many key characteristics that make them ideal organisms for detecting and evaluating contaminants in the environment. The conventional biomonitoring strategies focus on investigating the occurrence of priority pollutants or specific chemical classes, such as Per and Poly-Fluoroalkyl Substances (PFAS). Thus, many organic micropollutants remain uninvestigated and the chemical exposure of biota species is not explored to a high extent.

In the framework of this study, otter samples gathered from the United Kingdom were analyzed to investigate the different accumulation profiles of chemicals in seven different tissues collected during postmortem of road-killed otters (muscle, liver, kidney, pelt, blood, fat and fecal samples), through wide-scope target analysis. The sample preparation was conducted by applying generic protocols for the extraction of LC- and GC- amenable compounds with different physicochemical properties,

whereas the analysis was conducted using complementary chromatographic techniques coupled to HRMS. For the detected analytes, the investigation of the simultaneous presence of transformation products was conducted using suspect screening.

In total, 91 micropollutants, from different chemical classes, were determined during the targeted analysis. The most abundant class of contaminants were pharmaceuticals, followed by plant protection products and PFAS. Pelt accumulated the highest number of compounds with 45 contaminants detected, most of which were pharmaceuticals, followed by liver samples with 42 compounds, the majority of which were PFAS. Therefore, pelt, which could be potentially acquired non-invasively, may be of interest for the systematic monitoring of polar and semi-polar chemicals (including pharmaceuticals) in other samples. Moreover, based on our results, liver and fecal samples seem to accumulate plant protection products.

#### **1.10.P-We062 Pilot study: Seasonal pesticide exposure and corresponding metabolomic changes identified by non-target analysis of plasma samples obtained from hares in Austria.**

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The population dynamics of the European brown hare (*Lepus europaeus*) have exhibited a consistent downward trend over the past few decades, disregarding isolated regional deviations. Although the reasons for this decline are complex, some factors like chemical pressure may not have been adequately addressed. The use of pesticides has become essential in agriculture, and despite strict authorisation procedures, it is often not known whether and to what extent adverse effects on non-target organisms occur. In this study, plasma samples from 42 brown hares across different regions in Austria were analysed using non-targeted liquid chromatography-tandem mass spectrometry. Data mining included screening for pesticides potentially applied in the regions sampled. The samples include specimens from autumn 2020 and summer 2021, illustrating a distinct seasonal exposure pattern regarding the active substances of the applied plant protection products, as well as respective transformation products. While no pesticides or degradation products were detected in any of the animals taken in autumn, at least one pesticide was identified in more than 50 % of the samples taken in summer. In total, eight active substances of plant protection products and five metabolites were identified in 18 animals, with some individuals displaying a simultaneous exposure of up to eight different substances. Further statistical analyses revealed significant differences in the metabolomes of contaminated and uncontaminated hares. These results highlight the acute seasonal pesticide exposure in European brown hares and offer a valuable foundation for in-depth research into the metabolic effects and potential health consequences of pesticide exposure in European hares.

#### **1.10.P-We063 A Spatial Assessment of Neonicotinoid Exposure in Common Goldeneyes in Finland**

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Neonicotinoids are widely used insecticides in agriculture and veterinary medicine. While the primary scientific and public concern has been on the effect of neonicotinoids on pollinator populations, these contaminants have also been linked to adverse effects in farmland-associated birds. However, recent research shows that birds not directly linked to agricultural areas are also exposed to neonicotinoids. Many important rivers flowing into the Baltic Sea run through agricultural areas and are thus potential transmitters of neonicotinoids to the aquatic environment. As Finland both continues to use neonicotinoids and has several declining populations of waterbirds, it is pressing to examine whether these species are exposed to neonicotinoids. Here, we chose to investigate the occurrence of neonicotinoids in the common goldeneye (*Bucephala clangula*) as this bird can be found throughout Finland and can act as a proxy for more endangered waterbird species as well as being a good model species for assessing contamination of the aquatic environment. We collected blood plasma samples from incubating female goldeneyes ( $n = 51$ ) in 2022 from five different regions of Finland (Tornio, Seinäjoki, Maaninka, Helsinki, and Velkua) to assess if these compounds are present at quantifiable concentrations in this species and whether there is a spatial pattern to the concentrations and profiles linked to land use. The five regions follow a north-south gradient and are either predominantly agricultural or urban as well as including both inland and coastal areas. The target analytes are imidacloprid, acetamiprid, clothianidin, thiacloprid, thiamethoxam, nitenpyram, and dinotefuran. The samples have all been extracted and are scheduled for instrumental analysis in December-January, so the results will be ready to present at SETAC Seville.

#### **1.10.P-We064 Exploring 46 Years of Temporal Trends and Interspecific Patterns of Perfluoroalkyl Substances (PFASs) in 4 Seabird Species from Canada's Pacific Coast**

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Spatial and temporal trends of perfluoroalkyl substances (PFASs) have been well documented in marine food webs in the Atlantic, Indian, and Arctic oceans. However, much less is known about long-term trends of PFASs in the North Pacific Ocean, a region that remains largely understudied in coastal North America and is often subject to long-range transport (LRT) of PFASs and other contaminants from various North American and Asian sources. Here, we examined longer-term temporal

trends (1973-2019) of 4 PFSA and 13 PFCAs, as well as stable isotopes of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ), in the eggs of 4 seabird species sampled along a nearshore-offshore gradient; double-crested cormorants (*Nannopterum auritum*), pelagic cormorants (*Urile pelagicus*), rhinoceros auklets (*Cerorhinca monocerata*), and Leach's storm-petrels (*Hydrobates leucorhous*) from the Pacific coast of British Columbia, Canada. PFOS was the most abundant PFSA (79-94%) detected, with the highest concentrations, on average, measured in auklet eggs (mean PFOS = 58 ng/g, range = 11-286 ng/g ww) collected from relatively remote colonies. PFUDA and PFTRI were the dominant long-chain PFCAs ( $\geq 30\%$  combined). The majority of PFSA (including PFOS) are statistically declining ( $p < 0.001$ ) in the eggs of all 4 species with half-lives ranging from 2.6-7.8 years. Concentrations of long-chain PFCAs exhibited a trajectory comprised of linear increases (primarily due to PFNA, PFUDA, and PFTRI) as well as second-order declines ( $p < 0.001$ ) in recent years, suggesting that the rate of uptake of these PFCAs is slowing or levelling off in our species. However, egg values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  across species were nonlinearly correlated with  $\ln\sum\text{PFAS}$ s, suggesting that diet and foraging behaviour in nearshore/offshore areas may have influenced temporal trends. Nonetheless, these trends are consistent with the voluntary ceased production of PFSA and their precursors by 3M circa 2000-2003 and are among the first from Canada to indicate a positive response to North American legislative restrictions (e.g. US EPA, Health Canada) on PFCAs from facility emissions and product content.

#### **1.10.P-We065 PFAS accumulation and associations with reproduction in songbirds living near a hotspot**

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The area around 3M in Zwijndrecht, Belgium, has been well characterized to contain exceptionally high PFAS concentrations due to historical and recent emissions, making it an ideal site to study wildlife toxicity. Reproductive effects of PFAS on birds have been primarily studied under laboratory conditions, and field studies on PFAS (other than PFOS) are still relatively scarce. If present, they provide contradictory results, suggesting species-specific differences. In this study, we determined PFAS concentrations in eggs and blood plasma of chicks of both great tit (*Parus major*) and blue tit (*Cyanistes caeruleus*) in the vicinity of this fluorochemical plant. Furthermore, we associated accumulated PFAS concentrations to various breeding success parameters, including hatching and fledging success, survival, clutch size, and egg shell thickness. Nest-building and breeding was followed-up at three sites (i.e., 3M, the adjacent nature reserve Blokkersdijk, and Vlietbos, a forest area approximately 1.5 km from 3M) during the breeding season of 2022. When three eggs were present in a nest, a random egg was collected. Hereafter, the aforementioned breeding success parameters were determined. Blood samples were taken 14d after hatching, and were pooled among all chicks within a clutch to obtain sufficient plasma for PFAS analysis. Our preliminary results (further analysis will be conducted early 2024) reveal differences in PFAS accumulation profiles between blood plasma and eggs. Perfluorooctane sulfonate (PFOS) was the dominant PFAS in the eggs, with concentrations ranging between 34.5 and 67916 ng/g ww, whereas both PFOS (bLOQ – 15448 mg/L) and perfluorobutane sulfonate (PFBS; 41.3 – 24385 mg/L) were dominant in the blood plasma. Concentrations tended to decrease with distance from the 3M site, which is in agreement with previous studies in the area. Based on the field observations and earlier studies, limited effects of PFAS on breeding success are expected in both species, despite the high PFAS concentrations, although this still has to be confirmed by further statistical analysis.

#### **1.10.P-We066 Assessment of Persistent Organic Pollutants (POPs) in Common Kestrel Eggs from Urban and Rural Areas in Rome, Italy**

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The common kestrel is a diurnal raptor undergoing a moderate decline and chemical pollution represent a well known stressor for this species. In this study we investigated the presence of Persistent Organic Pollutants (POPs) in common kestrel eggs from urban (Aniene Park) and rural (Castel di Guido) locations in Rome, Italy. These locations represent diverse environmental conditions, offering insight into potential POPs exposure.

Gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) and low-resolution tandem mass spectrometry (GC-MS/MS) techniques were used for the quantification of selected POPs (OCPs, PCBs, PBDEs and PCDD/Fs). Our findings showed a varied concentration range of  $\sum\text{POPs}$  (17.4 to 975 ng/g ww) in common kestrel eggs, significantly higher in Aniene Park compared to Castel di Guido. PCBs dominated the POPs profile in both locations, followed by OCPs and then PBDEs, with low levels of PCDD/Fs. A higher contribution of OCPs to total POPs was found in Aniene Park.

Urban environments exhibited higher concentrations of  $\sum\text{DDTs}$ ,  $\sum\text{endosulfan}$ , and PeCB. Particularly, pp'-DDE emerged as a significant contributor to  $\sum\text{DDTs}$  and OCPs in both locations. HxCB and  $\sum\text{HCHs}$  depicted relatively high contributions, particularly in Castel di Guido. Isomeric analysis of  $\sum\text{HCHs}$  revealed notable differences between locations: in Castel di Guido, the  $\alpha$  and  $\gamma$  isomers displayed similarity, contrasting with the significant contribution of the  $\beta$  isomer in Aniene Park.  $\sum\text{endosulfan}$  showed a minimal presence. Concerning industrial and non intentional POPs, PCBs, PBDEs, PCDD/Fs, and PCDFs showed significantly higher median values in Aniene Park compared to Castel di Guido. No significant differences were observed for  $\sum\text{PCDDs}$ . The PCBs profile in both locations was dominated by PCB-118, followed by PCB-153. In the case of PBDEs, PBDE-153 was the predominant compound in both locations. However, PBDE-99 emerged as the subsequent prominent compound in Aniene Park, while PBDE-209 held that position in Castel di Guido. PCDD/F profiles varied between locations: in Aniene Park, 12378-PeCDF dominated, followed by 2378-TCDF, while in Castel di Guido, an equal dominance was seen for 2378-TCDF and 23478-PeCDF.

This study provides crucial insights into the prevalence and distribution of POPs in common kestrel eggs across urban and rural areas, emphasizing the need for continued monitoring and targeted mitigation strategies to safeguard avian populations in diverse habitats.

#### **1.10.P-We067 Difference in Toxicokinetics and Maternal Transfer between Lipophilic and Proteinophilic Halogenated organic pollutants in Laying Hens**

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Halogenated organic chemicals such as PCBs, PBDEs, DPs, and PFASs are pollutants with high environmental concerns. PCBs, PBDEs, and DPs are traditional lipophilic POPs while PFASs are well known as proteinophilic chemicals. BDE209 also exhibited some extent protein binding in biota. In the present study, the toxicokinetics and maternal transfer of PCBs, PBDEs, DPs, and PFCAs (C4-C18) in laying hens were investigated by exposing hens to spiked food. The highest uptake efficiencies were found for PCB congeners (>90%) and the lowest were found for BDE209 (21%). The uptake efficiencies of long chain PFACs (C>9, 76%-91%) were comparable with those of tetra-BDE to hex-BDE congeners (70%-91%) but were significantly higher than those of short chain PFACs (C<8, 30%-50%). The depuration rate (kd) of PFCAs were higher than those for PCB, PBDEs, and DPs and an inverted "U" relation between kd and carbon chain was found while a "V" relation was found between kd and log KOW for other chemicals. The bioaccumulation curves of PFACs can be divided into three groups (C4-C8, C9-C16, and C18) and other chemicals can be divided into two groups (all PCB and PBDE congeners without BDE209 as a group and BDE209 and DPs as a group). Adipose are main organ where all PCB and PBDE congeners without BDE209 deposited while BDE209 and DPs prefer to deposit in heart and muscle tissues. Yolk and brain are main organ/tissues where PFACs deposited and a tissue-specific affinity to specific carbon-chain PFCAs such as C9 for serum and liver, C18 for muscle, C14 for brain was observed. When different maternal tissues were chosen to calculate maternal transfer potential of chemicals, the correlation between calculated maternal transfer potential and chemical property (log KOW or carbon chain length) exhibited various, which indicated single maternal tissues is inappropriate to assess the maternal transfer potential of chemicals. The percentage of chemicals in egg to total chemicals in maternal body indicated that the maternal transfer potential of PFCAs (>80%) were significantly higher than those of BDE209 and DPs (about 30%) and the lowest maternal transfer potential were found for PCB and PBDE congeners without BDE209 (<10%). Exposure status (uptake or depuration phase) also affects the maternal transfer potential of chemicals. The results implied that lipophilic chemicals have high bioaccumulation but lower maternal transfer potential and vice versa for proteinophilic chemicals.

#### **1.10.P-We068 Carbamazepine Acts as Endocrine Disruptor of the Thyroid System in the Amphibian *Xenopus laevis***

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Carbamazepine (CARB) is a widely used pharmaceutical to treat epilepsy and neuropathic pain and furthermore as adjunctive medication of schizophrenia and bipolar disorder. Due to its common medication CARB is excreted at high concentrations and ends up via sewage treatment plants in surface waters reaching levels in the range of µg/L. CARB is known to affect hepatic enzymes and herewith it has been suggested to affect the thyroid system via an increased clearance rate of thyroid hormones (TH) by the liver. In order to assess the thyroid disrupting potential of CARB the amphibian metamorphosis assay (AMA) according to OECD test guideline 231 has been performed using CARB concentrations of 5.44, 54.4 and 544 µg/L. The AMA comprises as endpoints mainly gross morphological parameters, such as staging and hindlimb length, and after 21 d at the end of exposure also thyroid histopathology. All gross morphological endpoints revealed only a significant inhibition for staging after 7 d at the highest CARB concentration. Thyroid histopathology, however, resulted in moderate but significant effects even at the lowest concentration of 5.44 µg/L. Therefore, the proposed combination of gross morphological endpoints and classical thyroid histopathology reveals a great sensitivity regardless the mode of action for endocrine disruption of the thyroid system because TH agonists have only minor effects on thyroid histology but affect strongly gross morphological endpoints. The results are in accordance with the hypothesis that CARB increases elimination of TH by hepatic enzyme stimulation and reveal a significant dose dependent inhibition of stage development after 7 d. However, at the end of exposure after 21 d no significant effects are found neither at staging nor at any further gross morphological endpoint suggesting that the endogenous endocrine counter measure can balance the induced moderate impairment of metamorphosis via stimulation of the thyroid as seen by histology. In addition, whole tadpoles and target tissues such as pituitary, thyroid, liver, tail and eye have been excised to process them for transcriptomics and gene expression of established thyroidal biomarkers to get a deeper insight concerning modes of action and the potential for detecting new sensitive biomarkers. Acknowledgement: Funding by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825753 (ERGO).

#### **1.10.P-We069 Acute Dermal Toxicity of Pesticides to Terrestrial Metamorphs of the Spotted Salamander (*Ambystoma maculatum*)**

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Most of the toxicological research on amphibians focuses on anurans and on aquatic rather than terrestrial stages. For terrestrial stages, birds are often used as surrogates. However, bird studies focus on dietary exposure, while dermal exposure of amphibians is likely the more important exposure route.

Therefore, the goal of this study was to assess dermal toxicity of five insecticides (resmethrin, terbufos, endosulfan, bifenthrin, flufenoxuron) and two herbicides (pendimethalin, triclopyr) to terrestrial life stages of spotted salamanders (*Ambystoma*

*maculatum*) using the up-and-down testing procedure. We estimated LD<sub>50</sub> values through dorsal skin application of an active substance dissolved in a small volume of solvent (acetone, propylene glycol or polyethylene glycol) for each pesticide (depending on its solubility).

We also compare our results with standard dermal mammalian (rat) data and with outcomes of a dermal acute estimation model, originally developed for anurans, extrapolating from fish toxicity and bioconcentration data.

Of the seven pesticides tested, four appeared to have significant toxicity (LD<sub>50</sub> < 1750 µg/g), while flufenoxuron and the two herbicides were non-toxic (LD<sub>50</sub> > 1750 µg/g). LD<sub>50</sub> values for bifenthrin and endosulfan ranged from 5.5 to 17.5 µg/g, depending on the solvent. LD<sub>50</sub> values for terbufos ranged from 9.3 to 55 µg/g, depending on solvent, while for resmethrin both acetone and polyethylene glycol LD<sub>50</sub> values were 310 µg/g. When comparing toxicity across solvents within the same pesticide, there was only one instance where acetone appeared to increase toxicity (propylene glycol *versus* acetone for terbufos).

Our results suggest that mammalian dermal LD<sub>50</sub> values may underestimate sensitivity for terrestrial salamanders for the insecticides resmethrin, bifenthrin, and endosulfan. The calculations for the dermal acute estimation model are ongoing. Our study fills a knowledge gap regarding dermal acute toxicity to terrestrial life stages of amphibians and will help in estimating potential risk to amphibians due to dermal exposure.

#### **1.10.P-We070 Incorporating Mechanistic Effect Models into the Risk Assessment for Amphibians and Reptiles**

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The incorporation of mechanistic effect models (MEMs) is a central aspect of developing an environmentally relevant framework for the environmental risk assessment of pesticides in amphibian and reptiles.

As information is currently scattered, there is need to assess the state-of-the-art of MEMs for amphibians and reptiles and define a roadmap, ensuring that model development prioritizes activities which improve the applicability of MEMs to risk assessment.

For this reason, a workshop was held in Osnabrück on September 15 and 16 at Osnabrück University, Germany.

It was found that the development of organism-level models for amphibians and reptiles is already in an advanced state, with multiple models being developed for Amphibians for the purpose of modelling whole life-cycle effects as well as plasticity in the timing of metamorphosis.

Population models for amphibians are in a less mature state and can currently only be applied to simplistic scenarios.

Population models for reptiles have already been applied in more complex contexts, such as in combination with food web models.

The adoption of MEMs for the application in risk assessment requires agreement between the model functioning and the specific use of models, as well as validation against data. This is in contradiction with the availability of data. However, this further motivates the use of models as tools for exploration of possible effects and behavior of systems. Models might thus be a useful tool to prioritize a limited set of scenarios which require empirical testing.

Regarding the applicability of models, we differentiate between short-term applicability (“fit for purpose”) and long-term perspectives. On the short term, organism-level models may be fit for purpose to support risk assessment, for example to extrapolate across exposure scenarios or integrate apical endpoints. On the long term, we expect population models to be invaluable tools for risk assessment, but solutions need to be developed to resolve the conflict between the need for validation and the availability of data.

Within PERIAMAR, we further aim to increase the feedback between risk assessors and model developers. Proposed actions include the conduction of a survey among risk assessment agents to acquire an overview of the most useful model applications in risk assessment. This could form the basis of highly targeted development processes.

#### **1.10.P-We071 Amphibians in the Agricultural Landscape - Risk Reduction in Plant Protection and Promotion of Populations**

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This poster presents risk mitigation measures (RMM) for the terrestrial life stages of amphibians that can be recommended independently of the authorization of plant protection products (PPP). These measures should reduce the risk of PPP exposure, promote amphibians in agricultural areas and compensate for negative effects of PPP at the population level.

With the help of a literature review and expert surveys, potential RMM were compiled and evaluated in terms of their effectiveness in protecting or promoting amphibians, their feasibility on a farm and their controllability. In particular, existing measures for ecological compensation and PPP risk reduction were evaluated, but changes in agricultural practice were also taken into account. A total of 30 measures would be conceivable in Switzerland. Of these, nine RMM are proposed for practical application, whereby forward-looking and constructive cooperation between agriculture and amphibian conservation

is important for their implementation. Some of the measures are already being implemented or promoted, but can be adapted even more specifically for amphibians based on this work. In principle, the effect can be increased by combining measures.

GIS analysis and modeling provided a scientific basis for the selection of locally adapted measures with the greatest effect on amphibian populations. Models for the dynamics of populations and metapopulations of amphibians indicated that measures for the protection of tadpoles and especially juveniles (after metamorphosis) appear to have the greatest protective effect on the population. Regionally, the creation of new breeding sites (with suitable terrestrial habitat) is the most efficient measure. At the local level, small structures in terrestrial habitats or on seasonal migration routes are considered to be effective. Furthermore, we analyzed the use of agricultural landscapes by amphibians on a national scale and show where populations potentially live on agricultural areas with pesticide use. This work provides the basic information on where and when which species and life stages can be best protected and how. RMM should be selected and implemented on a species-specific basis. For example, a field study observed how natterjack toads used arable land as terrestrial habitat, as they prefer open ground. Biodiversity promotion areas with open soils would therefore be a promising enhancement measure to promote natterjack toads.

#### **1.10.P-We072 Evaluating Pesticide-Induced Risks to Amphibians in Agricultural Waters: A Case Study in Arroyo Pergamino, Argentina**

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The evaluation of the risks that pesticides pose to amphibians has become increasingly important, given the ongoing decline in amphibian populations. This issue is particularly pressing in countries such as Argentina, where agricultural production is predominant and large extensions of land are treated with pesticides many times per year. Amphibians are highly dependent on water bodies, and their ability to avoid pesticide exposure from contaminated water sources is limited due to habitat loss and fragmentation. Here, we evaluate the environmental risk of pesticides for amphibians in Arroyo Pergamino, a medium-sized stream that flows through the heart of the agricultural Pampa Region in Argentina. To do so, comprehensive pesticide residue data obtained from a year-long monitoring study were used. Pesticide data were obtained in four locations along the stream. These values were compared to toxicity data of the substances detected in the water, both for native and non-native amphibian species. When sufficient toxicity data was available, amphibian-specific acute and chronic predicted no effect concentrations (PNEC) were calculated by dividing the Lethal Concentration 50 (LC50 within 96 hours) and No Observed Effect Concentration (NOEC within 21 days) of the most sensitive amphibian species by an assessment factor (AF) of 10. Then, we calculated Risk Quotients (RQ) by dividing measured environmental concentrations of each single substance by their respective PNEC. To determine the risk threshold derived from acute toxicity data, we used the maximum measured concentrations of each site, while for the risk derived from chronic toxicity data, we used concentration means of the residues found in the monitoring studies. We used RQ to rank pesticides based on single substance risk and their contribution to mixture risk. Besides, we aimed to identify native amphibian species potentially vulnerable to pesticides detected in the stream. The result revealed that pesticides detected in Arroyo Pergamino pose a risk to amphibian health. However, the current lack of toxicity data for non-model species presents challenges in providing a definitive assessment across various species and regions. Despite this limitation, the results of this evaluation are expected to serve as a proof-of-concept risk assessment for amphibians inhabiting waters affected by agricultural activities.

#### **1.10.P-We073 Risk assessment screening step for dermal toxicity in terrestrial phase amphibians**

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Although currently considered covered by surrogate bird and mammal Environmental Risk Assessment (ERA) schemes, concerns for amphibian risk assessment have arisen regarding dermal exposure to pesticides in the terrestrial phase, primarily due to the high permeability of their skin and the lack of a protective layer such as fur or feathers. This, coupled with the uncertainties regarding differences in amphibian toxicity when compared to birds and mammals are currently considered the main hindrances for addressing amphibian-specific risks of plant protection products.

Toxicity data for terrestrial life-stages of amphibians are lacking. While this is true both for acute and chronic toxicity, some acute data are available which can be compared to bird, mammal, or fish data. Based on this information, extrapolation methods have been described with which acute toxicity endpoints (LD<sub>50</sub>) can be extrapolated for adult (terrestrial) amphibians. Similarly, although considered of high importance, dermal exposure data in amphibians are very scarce and limited to a few compounds. However, exposure can be estimated using toxicokinetic models, which allow to predict body burdens resulting from dermal exposure due to contact with contaminated surfaces, such as soil.

By combining these two methods, a screening assessment for dermal exposure can be described based on already available regulatory data. The goal of such a screening step would be to identify plant protection products of low and high concern for amphibians, in order to prioritize risk assessment efforts. At the same time, extrapolation and modelling approaches will help in reducing unnecessary animal testing and suffering, while allowing risk evaluation despite the lack of amphibian-specific data.

Here, we present a screening approach in which acute risk to amphibians was evaluated for selected pesticides based on the abovementioned toxicity and exposure estimation methods.

#### **1.10.P-We074 Suggestion of Focal Species of Amphibians in the Context of Environmental Risk Assessment of Pesticides in Brazil**

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Amphibians have the highest proportion of threatened species among vertebrates and exposure to pesticides has been identified as one of the potential causes for population declines of several species. This study aimed to identify a potential focal species of amphibians that could be used in the Environmental Risk Assessment (ERA) of pesticides in Brazil. The appraisal was based on (1) exposure potential due to the ecological characteristics of the species and the overlap of their distribution with agropastoral lands and (2) their relative sensitivity to pesticides, compared to fish. For step 1, distribution maps for 1106 amphibian species were constructed. Then, the maps were superimposed with Brazilian maps of agropastoral lands, totaling 805 species possibly occurring in such areas. Later, data was filtered to consider only highly abundant species with no associated taxonomic issues and widely distributed in Brazil – resulting in 76 species. Ecological data were then collected for these species and a Non-metric Multidimensional Scaling was carried out, resulting in 30 possible focal species. For step 2, systematized searches on the USEPA “Ecotox Knowledgebase” database and in Web of Science and Google Scholar platforms were performed for ecotoxicological studies with herbicides on larval stages of amphibians in Brazil. Among the 115 active substances of herbicides registered in the country, only 16 presented LC50 data for native species of amphibians and 17 for model species. LC50 values of the same herbicides were also compiled for model fish species. LC50 for fish and amphibians were then combined to construct Species Sensitivity Distribution (SSDs) curves. Due to data availability, SSDs were constructed for only seven herbicides. Amphibian species showed less sensitivity to acetochlor and ametryne than fish. For atrazine, glyphosate, glyphosate-isopropylamine salt, picloram, and 2,4-D, the species *Physalaemus centralis*, *P. cuvieri*, *P. nattereri*, and *P. albonotatus* were more sensitive than the standard fish species *Oncorhynchus mykiss*. The evident sensitivity of *Physalaemus* spp., associated with their occurrence on the focal species list suggested in Step 1 indicates that this genus contains species potentially sensitive to the toxicological effects of pesticides, which could be important vertebrate bioindicators for neotropical areas. This study is a pioneer in indicating a possible focal species for the ERA of pesticides for amphibians in Brazil.

#### **1.10.P-We075 Mass spectrometry imaging of the developing amphibian brain as a tool to discover bioindicators of developmental neurotoxicity.**

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Neurodevelopment is highly conserved across animal species and is a vulnerable choreography of events that unfolds to construct highly complex organs. Exposure to neurotoxic substances can lead to failure in proper spatial and temporal organization of neural tissues. Despite this, the current guidelines are insufficient to properly identify chemicals that are toxic for the developing brain.

Amphibians are exceptional alternative model organisms for uncovering neurotoxicity during postembryonic development because the reorganization of a tadpole brain during metamorphosis has direct parallels to the developing human brain during the last trimester and the first weeks of life. Herein we are utilizing a representative of true frogs, the cosmopolitan tadpole model American bullfrog (*Rana [Lithobates] catesbeiana*) and mass spectrometry imaging to discover bioindicators of neurodevelopmental toxicity. Mass spectrometry imaging is a unique technology that adds spatial information to mass spectrometry analysis, allowing mapping of specific biomolecules in the tissue. This is particularly relevant in the brain, where different tissue regions have different functions.

We examined three distinct postembryonic life stages: premetamorphic tadpoles (Gosner stage 31-33), tadpoles at metamorphic climax (Gosner stage 41-44) and froglets (Gosner stage 46). Cryosections of the head, including the olfactory system and brain, were obtained, and analyzed by Matrix-Assisted Laser Desorption/Ionization coupled to a Time-of-Flight mass spectrometer (MALDI-TOF) for non-targeted analysis focussed on lipid metabolites. Brain lipids are of particular interest as they are critical for the health of the brain and its activities such as structural development, neurogenesis and in myelin sheath formation. We found that several lipids varied in their spatial location and relative concentration between the developmental stages. These compounds represent candidates for neurodevelopmental bioindicators and may serve as valuable, quantifiable key events in future identification of chemicals causing developmental neurotoxicity.

### 1.10.P-We076 Aquatic Risk Assessment of Pesticides in Swiss Habitats of National Importance

*Etienne Vermeirssen<sup>1</sup>, Marion Junghans<sup>1</sup>, Christoph Moscher<sup>2</sup> and Lukas Kohli<sup>3</sup>, (1)Swiss Centre for Applied Ecotoxicology, Switzerland, (2)Inter Cantonal Laboratory, Switzerland, (3)Hintermann & Weber, Switzerland*

In 2017, Switzerland adopted an action plan covering plant protection products (PPP) with one of its aims: a reduction of risks that PPP pose to the aquatic environment. Long-running monitoring programmes provide good data coverage of streams in areas with agricultural and urban land use. However, similar investigations are lacking for PPP in smaller standing waters, particularly those classified by law as habitats of national importance which are often listed as spawning areas for amphibians. In our study, listed habitats were included for preselection based on proximity to agricultural land use using a 2 x 2 km<sup>2</sup> grid. Selected areas were then classified in low, medium and high risk categories based on i.a. the proportion to agricultural land use and their proximity to such land use. Six water bodies, two of each risk category, were sampled in 2020; six further water bodies were sampled in 2021. At each location, grab water samples were taken on a monthly basis for either 6 months in 2020 or 8 months in 2021. Chemical analyses of samples encompassed some 100 compounds, mainly PPP, transformation products, biocides and pharmaceuticals. Observed concentrations were evaluated against chronic and acute water quality criteria provided in the Swiss Water Protection Ordinance (WPO) as well as those available from own research. Results show that in half of the studied water bodies at least one compound exceeded either chronic or acute WPO criteria. When including the wider set of criteria, two further sites showed exceedances. Exceedances were not linked to the site's risk category. Main drivers for exceedances were pyrethroid insecticides and the maximal exceedance of WPO criteria was 25 fold for cypermethrin. A chronic water quality criterion derived for deltamethrin of 1.7 pg/L (not yet included into the WPO) was exceeded at most sites with a maximum exceedance of ca. 1200 fold. Although populations of amphibians were not specifically monitored for this study, the multiple and partially large exceedances of water quality criteria indicate that effects on aquatic wildlife cannot be excluded. Even if amphibians may not be directly affected by the observed concentrations of monitored compounds, effects on biodiversity or food webs may impact amphibian populations. The observed significant occurrence of PPP in the studied habitats warrants further study by expanding the studied locations and investigating PPP entry pathways to address possible mitigation measures.

### 1.11 New Approach Methodologies (NAMs) – Robust Predictions for Addressing Regulatory Challenges in the Field of Ecotoxicology

#### 1.11.P-Th064 Machine Learning-based Prediction of Fish Acute Mortality: Implementation, Interpretation, and Regulatory Relevance

*Christoph Schuer<sup>1</sup>, Lilian Gasser<sup>2</sup>, Fernando Perez-Cruz<sup>2</sup>, Kristin Schirmer<sup>1</sup> and Marco Baity-Jesi<sup>3</sup>, (1)Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland, (2)Swiss Data Science Center, Switzerland, (3)Siam, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland*

The use of historic data and chemical properties for the prediction of ecotoxicological outcomes has a long tradition within the realm of ecotoxicological research, for the most part in the form of quantitative structure-activity-relationship (QSAR) models. However, recent developments of machine learning (ML) in terms of accessibility, software packages, and computational power have led to them presenting a viable option for the inclusion in predictive ecotoxicology. A significant advantage of these methods lies in their ability to integrate data beyond chemical properties and descriptors, thus enabling researchers to include species-specific and experimental data, expanding their applicability beyond limited species and chemical domains. Our work aims to assess the potential and limitations of applying machine learning to predict ecotoxicological outcomes in fish. After data curation we coupled several state-of-the-art ML regression models with 6 molecular representations and applied this toolbox to a dataset focused on 140 fish species and 1,905 chemicals. The two decision-tree-based models RandomForest and XGBoost performed best and the linear-regression-based LASSO model performed worst. Comparisons of the 6 molecular representations did not yield a definitive winner, even though the best performing combination was the MACCS fingerprint in conjunction with the XGBoost model. Globally, this combination was able to predict the log-transformed LC50 within one order of magnitude, while local model performances varied more widely. Additionally, we performed an analysis of residuals and feature importances. These led to the conclusion that chemical properties influence the model performance the most, while taxonomic features do not consistently show up as important. However, if we train models only based on the most important features, those perform markedly worse than those based on the whole feature space. Following this broad modeling approach, we adapted the workflow towards optimized predictions of single fish species, mimicking the OECD test guideline used for regulatory hazard assessment. By limiting the taxonomic space we achieved better model performances and make the model output transparent and discuss it through the lens of hazard assessment.

#### 1.11.P-Th065 Connecting the Dots: A Bottom-Up Approach to Link Liver Fibrosis with Key Events through Text Mining and Topic Modeling in AOP Development

*Achilleas Karakoltzidis, Ilias Frydas, Spyros Karakitsios and Dennis Sarigiannis, Aristotle University of Thessaloniki, Greece*  
A novel methodology for the construction of AOPs is introduced, with liver fibrosis serving as the paradigm. Our approach integrates cutting-edge models for biomedical text mining and employs two distinct techniques for topic modeling, namely LDA and BERTopic. The utilization of two different algorithms enabled a cross-validation between the topics obtained from the two different methods. These techniques enhance the understanding of complex relationships within biological processes, thus providing a systematic and data-driven approach for constructing AOPs. Following a literature review, a set of keywords related to liver fibrosis was initially identified. Instances of the keywords included Cytokines, Ehr, chemokines, among others. Afterwards, a search on PubMed was conducted, collecting all available abstracts. A total of 195748 abstracts were collected.



Afterwards duplicates were removed and the total number of abstracts remaining for analysis was 191029. The abstracts organized in tabular form from. They were tokenized and cleaned by removing stop words and lemmatized. After processing the abstracts, the topic modeling results were organized into a network. This facilitated the identification of nodes and connections between the significant factors associated with liver fibrosis. Examples encompass liver diseases like HVB/C, inflammation, exposure to specific chemicals, oxidative stress, and cellular death, among others. It is worth noting that many literature reports have documented the presence of patients suffering from both liver fibrosis and insulin resistance. The identified topics will enable us to refocus the literature review on specific points, intentionally constructing an AOP. Consequently, the next phase entails acquiring new articles based on keywords identified through topic modeling. The collected publications will undergo two distinct text mining methods: biological entity recognition, and biological entity extraction. The goal is the systematic construction of an AOP for liver fibrosis, employing a bottom-up approach. Simultaneously, articles categorized with significant topics relevant to the present study will be included in the subsequent analysis. It is noteworthy that in the ensuing step, after reapplying topic modeling to the newly collected articles and preserving the relevant ones, is to proceed to a comprehensive full text analysis.

#### **1.11.P-Th066 High-Throughput Transcriptome in Zebrafish Embryos towards Adverse Outcome Pathway Network-Based Screening of Environmental Neurotoxicants**

*Pu Xia<sup>1</sup>, Yuwei Xie<sup>2</sup>, John K. Colbourne<sup>3</sup>, Luisa Orsini<sup>3</sup>, John Giesy<sup>1</sup> and Xiaowei Zhang<sup>4</sup>, (1)University of Saskatchewan, Canada, (2)Nanjing Institute of Environmental Sciences, China, (3)School of Biosciences, University of Birmingham, United Kingdom, (4)Nanjing University, China*

The increasing presence of neuroactive chemicals in the environment poses a significant threat to the developing nervous systems of both humans and wildlife. To address this concern, there is an urgent need for new methodologies that are fast, cost-effective, and accurate in testing developmental neurotoxicity. High throughput screening (HTS) approaches have emerged as a potential solution. Current *in vitro* assays focus on targeted biological pathway responses at the biomolecular level but are limited by known biological endpoints, which account for less than 3% of neurodevelopment-related aspects. *In vivo* assays, particularly using small animals like zebrafish, offer high-throughput screening of phenotypic profiles but lack the ability to elucidate the molecular mechanisms underlying neurotoxicity.

Omics approaches, such as high-throughput transcriptomics (HTT), provide a genome-scale understanding of biological pathways affected by chemical exposure. However, interpretation of omics responses into apical effects has traditionally relied on expert-based manual explanations. The Adverse Outcome Pathway (AOP) framework offers a promising approach to bridge the gap between molecular-level perturbations and regulatory concerns' apical effects.

This study incorporates zebrafish transcriptomics and behavioral assays to develop an AOP network-based approach for assessing environmental neurotoxicants. Dose-dependent transcriptomics across multiple time points were conducted to identify neurotoxic modes of action for model neurotoxicants at specific developmental stages. The study aims to evaluate the AOP network-based approach's ability to qualitatively identify neurotoxic modes of action and quantitatively derive genomics-based points of departure comparable to behavior-based points of departure. Additionally, the research explores the potential of the AOP network-based approach for screening neurotoxic chemicals using dose-dependent transcriptomics in zebrafish embryos.

#### **1.11.T-01 Commission Roadmap towards Phasing out Animal Testing for Chemical Safety Assessments**

*Georg Streck, European Commission - DG GROW, Belgium*

On 25 July 2023, the European Commission published a Communication, with which it committed to develop a roadmap towards ultimately phasing out animal testing for chemical safety assessments. The purpose of this presentation is to provide an overview of the process of the development of the roadmap and to outline possibilities for stakeholders from the research community, industry and non-governmental organisations to provide input to the work.

The European Union has a long-standing policy that aims to phase out all animal use for research and for regulatory purposes in the EU, if and whenever it is scientifically possible. In the last years, the question of how to reach this ultimate goal has gained more and more political attention, e.g. as evidenced by a resolution of the European Parliament. Notwithstanding the progress made in developing non-animal methods, an answer to the question has not yet been found and animal testing is still needed, e.g. to assess chemicals for complex toxicities. The roadmap has the purpose to outline the path towards phasing out animal testing for chemical safety assessments.

The cornerstone of the roadmap is a plan outlining of how to phase out animal testing for each area of concern (toxicity, (eco-)toxicological endpoint), starting with a gap analysis. For some areas of concern, short-term solutions are available (e.g. fish acute toxicity, bioaccumulation), while for others long-term action points might be necessary to define data requirements in legislation differently so that non-animal methods can be used to fulfil the ultimate regulatory goal of protecting human health and the environment.

The roadmap will also seek for solutions of how to accelerate the validation of methods. In addition, there is a need to speed up the regulatory acceptance of methods. In this context, the Commission is analysing the need and feasibility of an expert

scientific committee to provide advice on the development and regulatory application of non-animal methods. This analysis goes along with analysing the strengths and weaknesses of the current landscape of advisory bodies on non-animal methods.

An exchange in particular with the scientific community is vital for developing the roadmap. The presentation will therefore outline how stakeholders can get involved in the shaping the path towards phasing out animal testing for chemical safety assessments.

#### **1.11.T-02 Building an alternative fish to improve current environmental risk assessment schemes for chemicals**

**Kristin Schirmer<sup>1</sup>**, Ksenia J Groh, PhD<sup>2</sup>, Melanie Fischer<sup>2</sup>, Jarno Hoekman<sup>3</sup>, Stephan Fischer<sup>4</sup>, Roman Li<sup>5</sup>, Bernhard Truffer<sup>6</sup>, Colette vom Berg<sup>7</sup> and Anže Županič<sup>8</sup>, (1)Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland, (2)Eawag - Swiss Federal Institute of Aquatic Science and Technology, Switzerland, (3)Utrecht University, Netherlands, (4)aQuaTox-Solutions GmbH, Switzerland, (5)aQuaTox-Solutions/Eawag, Switzerland, (6)Eawag/Utrecht University, (7)Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland, (8)National Institute of Biology, Slovenia

Because of their importance in aquatic ecosystems and as human food source, fish receive great attention in the environmental risk assessment of chemicals. However, current animal-centric testing paradigms cannot keep pace with the demands of chemical safety assessment and the societal desire to reduce or replace animal testing. It is, therefore, our vision to build an alternative fish: a modular assemblage of fish cell line-based assays, alone or combined with computational models. Such an alternative fish represents a new method that promises to overcome some of the limitations of the traditional testing approach and holds tremendous opportunity for industrial innovation. Yet, validation of the new approaches takes years and uptake is slow. We, therefore, argue that regulatory routines and procedures, as well as industrial innovation practices, need to be considered in the design of such alternative approaches to accelerate their acceptance and implementation into practice.

Pursuing this vision, we have demonstrated that it is possible to use a rainbow trout (*Oncorhynchus mykiss*) cell line to predict the impact of chemicals on two *in vivo* apical endpoints: short-term survival and growth. Short-term survival is predicted using the RTgill-W1 cell line assay, which has reached global acceptance as ISO standard 21115 and OECD Test Guideline 249; it hence comprises the first complete module of our envisioned alternative fish. Prediction of reduced fish growth measures cell proliferation as a proxy for fish cell number/weight. We further expand these concepts to modules for neuro- and reproductive toxicity, and to subcellular markers with a documented link to adverse outcomes in fish, and have implemented an array of computational models to extrapolate cell line-derived information to predict *in vivo* outcomes.

We propose to co-develop the alternative fish platform with the relevant stakeholders from the start by applying a socio-technical perspective. The goal is to create a common understanding of the challenges and opportunities and to identify the needs and capabilities of different stakeholders to accelerate uptake.

Using the alternative fish framework as a test case, we aspire to initiate a paradigm shift in the socio-technical testing regime from single animal replacement options and assay-by-assay validation to a flexible framework that enables a more rapid transition towards the replacement and reduction of animals in environmental risk assessment.

#### **1.11.T-03 Integration of Endocrine Modalities into an Existing Mechanism of Action-based In Silico Scheme for use in Environmental Risk Assessment**

**James Firman<sup>1</sup>**, Franklin Bauer, PhD<sup>2</sup>, Geoffrey Hodges<sup>3</sup>, Gaspard Level<sup>2</sup>, Bruno Campos<sup>3</sup>, Jayne Roberts<sup>3</sup>, Steve Gutsell<sup>3</sup>, Homa Basiri<sup>1</sup>, Paul Thomas<sup>2</sup> and Mark Cronin<sup>1</sup>, (1)Liverpool John Moores University, United Kingdom, (2)KREATiS SAS, France, (3)Unilever - Safety and Environmental Assurance Centre (SEAC), United Kingdom

The adoption of *in silico* methodologies for the inference of toxic potential has been advanced as a means through which challenges associated with data scarcity, as relates to chemical risk assessment within the environmental context, might be addressed. Previously, we have reported upon the development of mechanism of action-centred, structural alert-based profiling/classification schemes (MechoA and Sapounidou-Firman), facilitating the assignment of likely mechanisms of toxic action in a variety of taxa. However, it is recognised currently that there is a paucity of alerts permitting the identification of substances which may be considered to act specifically upon the endocrine system.

As such, we have sought to expand the scope of these tools so that they might integrate alerts covering many further endocrine-associated pathways. An account of progress to-date is presented – incorporating description of processes behind the preliminary identification of molecular initiating event (MIE) targets, and the subsequent organisation and placement of these within the existing scheme. To this end, a tiered system of classification was adopted, grouping MIEs in accordance with shared downstream key events. More than 25 sites of action have been identified as forming suitable candidates for inclusion. These range from key hormone binding receptors (e.g., androgen, thyroid), to enzymes essential within hormone synthesis (e.g., 5 $\alpha$ -reductase, thyroperoxidase), to proteins mediating hormone distribution (e.g., transthyretin). Represented within this number are pathways relevant in mammalian, fish and arthropod species.

With reference to selected MIE targets, we further illustrate application of workflows assisting the derivation of the novel structural alerts. Incorporated within are stages spanning both the sourcing of appropriate data, and the subsequent identification of key chemical features selectively associated with activity.

#### **1.11.T-04 Predicting Aquatic Toxicity of Surfactants Using Simulated Coarse-Grained Membrane-Water Coefficient Derived QSARs**

*Andrea Gredelj*<sup>1,2</sup>, *Jayne Roberts*<sup>1</sup>, *Elin Barrett*<sup>3</sup>, *Eoin Kearney*<sup>4</sup>, *Nicola Haywood*<sup>1</sup>, *Mark Miller*<sup>4</sup> and *Geoffrey Hodges*<sup>1</sup>, (1)Unilever - Safety and Environmental Assurance Centre (SEAC), United Kingdom, (2)Norwegian Geotechnical Institute (NGI), Norway, (3)Safety and Environmental Assurance Centre, Unilever Research, United Kingdom, (4)Department of Chemistry, Durham University, United Kingdom

Recent developments in regulatory landscapes have been encouraging a shift away from generating *in vivo* data towards the use of *in vitro* and *in silico* approaches to support New Approach Methodologies (NAM) based non-animal alternatives. Quantitative Structure-Activity Relationships (QSAR) models are well-established within regulatory guidelines as a means for predicting (eco)toxicity endpoints. The octanol-water partitioning coefficient ( $K_{ow}$ ) is commonly applied as a predictor variable for (eco)toxicity, but its applicability for surfactants has been criticised based on experimental challenges in its determination and the lack of biological relevance. The membrane-water distribution coefficient ( $D_{mw}$ ) has been demonstrated previously as a potentially suitable alternative for surfactants, with established experimental methods appropriate for surfactants (and ionisable chemicals) as well as its applicability as a bioaccumulation proxy.

Here, the application of  $D_{mw}$  in building QSARs for predicting (eco)toxicity of fish and *Daphnia* species will be demonstrated for the group of hydrocarbon- and perfluorocarbon-based anionic surfactants.  $D_{mw}$  values were generated by the coarse-grained simulation method, which was successfully developed further for both of these surfactant groups. These predicted values were compared with existing and newly generated experimental  $D_{mws}$ , demonstrating a good fit for anionic surfactants with different functional groups and backbones.

While developed QSARs have high relevance for use in Environmental Risk Assessment (ERA) in both regulatory and non-regulatory applications, the new simulation method and its validation for these and other ionisable chemical groups provides another *in silico* approach that will make  $D_{mw}$  predictions more reliable and readily available.

Furthermore, to our knowledge, those are the first QSARs that incorporate different anionic surfactant groups into a single QSAR and the first (eco)toxicity QSAR derived for different (legacy and novel) perfluorinated surfactants that are based on membrane-water partitioning as an (eco)toxicity proxy. This work also presents a step forward towards increasing the application of *in silico* NAMs for surfactants which are in general high tonnage, high usage types of chemicals and commonly registered under REACH.

#### **1.11.P New Approach Methodologies (NAMs) – Robust Predictions for Addressing Regulatory Challenges in the Field of Ecotoxicology**

##### **1.11.P-Th064 Machine Learning-based Prediction of Fish Acute Mortality: Implementation, Interpretation, and Regulatory Relevance**

*Christoph Schuer*<sup>1</sup>, *Lilian Gasser*<sup>2</sup>, *Fernando Perez-Cruz*<sup>2</sup>, *Kristin Schirmer*<sup>1</sup> and *Marco Baity-Jesi*<sup>3</sup>, (1)Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland, (2)Swiss Data Science Center, Switzerland, (3)Siam, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland

The use of historic data and chemical properties for the prediction of ecotoxicological outcomes has a long tradition within the realm of ecotoxicological research, for the most part in the form of quantitative structure-activity-relationship (QSAR) models. However, recent developments of machine learning (ML) in terms of accessibility, software packages, and computational power have led to them presenting a viable option for the inclusion in predictive ecotoxicology. A significant advantage of these methods lies in their ability to integrate data beyond chemical properties and descriptors, thus enabling researchers to include species-specific and experimental data, expanding their applicability beyond limited species and chemical domains. Our work aims to assess the potential and limitations of applying machine learning to predict ecotoxicological outcomes in fish. After data curation we coupled several state-of-the-art ML regression models with 6 molecular representations and applied this toolbox to a dataset focused on 140 fish species and 1,905 chemicals. The two decision-tree-based models RandomForest and XGBoost performed best and the linear-regression-based LASSO model performed worst. Comparisons of the 6 molecular representations did not yield a definitive winner, even though the best performing combination was the MACCS fingerprint in conjunction with the XGBoost model. Globally, this combination was able to predict the log-transformed LC50 within one order of magnitude, while local model performances varied more widely. Additionally, we performed an analysis of residuals and feature importances. These led to the conclusion that chemical properties influence the model performance the most, while taxonomic features do not consistently show up as important. However, if we train models only based on the most important features, those perform markedly worse than those based on the whole feature space. Following this broad modeling approach, we adapted the workflow towards optimized predictions of single fish species, mimicking the OECD test guideline used for regulatory hazard assessment. By limiting the taxonomic space we achieved better model performances and make the model output transparent and discuss it through the lens of hazard assessment.

### 1.11.P-Th065 Connecting the Dots: A Bottom-Up Approach to Link Liver Fibrosis with Key Events through Text Mining and Topic Modeling in AOP Development

*Achilleas Karakoltzidis, Ilias Frydas, Spyros Karakitsios and Dennis Sarigiannis, Aristotle University of Thessaloniki, Greece*

A novel methodology for the construction of AOPs is introduced, with liver fibrosis serving as the paradigm. Our approach integrates cutting-edge models for biomedical text mining and employs two distinct techniques for topic modeling, namely LDA and BERTopic. The utilization of two different algorithms enabled a cross-validation between the topics obtained from the two different methods. These techniques enhance the understanding of complex relationships within biological processes, thus providing a systematic and data-driven approach for constructing AOPs. Following a literature review, a set of keywords related to liver fibrosis was initially identified. Instances of the keywords included Cytokines, Ehr, chemokines, among others. Afterwards, a search on PubMed was conducted, collecting all available abstracts. A total of 195748 abstracts were collected. Afterwards duplicates were removed and the total number of abstracts remaining for analysis was 191029. The abstracts organized in tabular form from. They were tokenized and cleaned by removing stop words and lemmatized. After processing the abstracts, the topic modeling results were organized into a network. This facilitated the identification of nodes and connections between the significant factors associated with liver fibrosis. Examples encompass liver diseases like HVB/C, inflammation, exposure to specific chemicals, oxidative stress, and cellular death, among others. It is worth noting that many literature reports have documented the presence of patients suffering from both liver fibrosis and insulin resistance. The identified topics will enable us to refocus the literature review on specific points, intentionally constructing an AOP. Consequently, the next phase entails acquiring new articles based on keywords identified through topic modeling. The collected publications will undergo two distinct text mining methods: biological entity recognition, and biological entity extraction. The goal is the systematic construction of an AOP for liver fibrosis, employing a bottom-up approach. Simultaneously, articles categorized with significant topics relevant to the present study will be included in the subsequent analysis. It is noteworthy that in the ensuing step, after reapplying topic modeling to the newly collected articles and preserving the relevant ones, is to proceed to a comprehensive full text analysis.

### 1.11.P-Th066 High-Throughput Transcriptome in Zebrafish Embryos towards Adverse Outcome Pathway Network-Based Screening of Environmental Neurotoxicants

*Pu Xia<sup>1</sup>, Yuwei Xie<sup>2</sup>, John K. Colbourne<sup>3</sup>, Luisa Orsini<sup>3</sup>, John Giesy<sup>1</sup> and Xiaowei Zhang<sup>4</sup>, (1)University of Saskatchewan, Canada, (2)Nanjing Institute of Environmental Sciences, China, (3)School of Biosciences, University of Birmingham, United Kingdom, (4)Nanjing University, China*

The increasing presence of neuroactive chemicals in the environment poses a significant threat to the developing nervous systems of both humans and wildlife. To address this concern, there is an urgent need for new methodologies that are fast, cost-effective, and accurate in testing developmental neurotoxicity. High throughput screening (HTS) approaches have emerged as a potential solution. Current *in vitro* assays focus on targeted biological pathway responses at the biomolecular level but are limited by known biological endpoints, which account for less than 3% of neurodevelopment-related aspects. *In vivo* assays, particularly using small animals like zebrafish, offer high-throughput screening of phenotypic profiles but lack the ability to elucidate the molecular mechanisms underlying neurotoxicity.

Omics approaches, such as high-throughput transcriptomics (HTT), provide a genome-scale understanding of biological pathways affected by chemical exposure. However, interpretation of omics responses into apical effects has traditionally relied on expert-based manual explanations. The Adverse Outcome Pathway (AOP) framework offers a promising approach to bridge the gap between molecular-level perturbations and regulatory concerns' apical effects.

This study incorporates zebrafish transcriptomics and behavioral assays to develop an AOP network-based approach for assessing environmental neurotoxicants. Dose-dependent transcriptomics across multiple time points were conducted to identify neurotoxic modes of action for model neurotoxicants at specific developmental stages. The study aims to evaluate the AOP network-based approach's ability to qualitatively identify neurotoxic modes of action and quantitatively derive genomics-based points of departure comparable to behavior-based points of departure. Additionally, the research explores the potential of the AOP network-based approach for screening neurotoxic chemicals using dose-dependent transcriptomics in zebrafish embryos.

### 1.11.P-Th067 Addressing the challenges of Acute Toxicity Hazard Classification using a non-animal Defined Approach

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The current regulatory landscape for assessing chemical safety is driven by a historic requirement for *in vivo* testing. Specifically, current regulatory frameworks for classification and labelling (C&L) and Persistence, Bioaccumulation and Toxicity (PBT) and very Persistent, very Bioaccumulative (vPvB), drive the need for aquatic toxicity studies involving fish, amphibians (for endocrine properties), birds (for pesticides) and mammals. However, this is incongruous with the current societal and ethical shift towards non-animal testing. In response to this circumstance, there are increasing signs of change in some regulatory landscapes such as REACH. For example, there have been significant efforts in recent years to reduce or

eliminate the number of vertebrates, such as fish, in regulatory assessments, by applying the 3Rs. Notably, there has been progress in opportunities to replace some *in vivo* tests with *in vitro* assays, such as RTgill-W1 (OECD TG 249) assay, or developing read-across, weight of evidence (WoE) and Integrated Approaches to Testing and Assessment (IATA) approaches to support chemical registration. However, to date there is no clear agreement on how to move these opportunities towards the greater use of non-animal New Approach Methodologies (NAMs) so that they could be fully integrated into regulatory frameworks such as EU Classification, Labelling and Packaging (CLP) and United Nations Globally Harmonized System of Classification and Labelling (UN GHS) frameworks.

Here we present a modular score-based *in silico* defined approach, which could be used to address the current requirements for *in vivo* acute aquatic toxicity under the CLP and UN GHS frameworks. The approach was developed using a curated, high-quality, literature based/publicly sourced dataset of existing *in vivo* and *in vitro* data, Mode of Action (MoA) assessment and predictions from several different *in silico* models to predict classifications of acute aquatic toxicity. Several case studies are presented which incorporate the defined approach prediction, physicochemical parameters and existing data to support the classification in a WoE.

### 1.11.P-Th068 Development of an Ontology-Driven In Silico Profiler for the Evaluation of Potential Endocrine Disruptors

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The identification of xenobiotics with potential to cause endocrine disruption is a process likely to draw heavily upon the use of new approach methodologies (NAMs). *In silico*, or computational, NAMs will be applied early in any screening process. This investigation describes the development of an *in silico* screening tool based upon known mechanisms of endocrine disruption. Specifically, we present here a novel ontology for endocrine disruption, categorising more than 25 molecular initiating events (MIEs), was used as its foundation. The ontology builds upon previous work categorising acute mechanisms of ecotoxicity, and allows for association of MIEs with species-specific adverse outcomes within well-defined chemical domains. To develop the profiler, data relating to the MIE were retrieved from publicly available resources such as Tox21 and ChEMBL. The data were curated and allocated in line with specific outcomes such as agonism, antagonism and inhibition. Chemical descriptors and properties were calculated, along with ToxPrint fingerprints. Subsequently, data were subject to chemoinformatic analysis using machine learning techniques, enabling groups of similar molecules to be identified. Approaches adopted included hierarchical clustering (based upon shared structural features) and the extraction of Murcko scaffolds. Collections of the grouped substances related directly to MIEs were considered to form the starting points for structural alert development. Each cluster and scaffold-sharing group is described fully in terms of the nature and type of data from which it is derived, the aspects of the applicability domain (such as the range of physico-chemical properties and types of molecular substituents) associated with it, and the overall confidence score (representing the numbers and quality of active data) which may be attributed. The *in silico* profiler will be made publicly available in the form of a computational workflow, the application of which will serve as a first-pass rapid screening tool to highlight possibility of compounds being associated with endocrine disruption MIEs. This could form the basis of a testing strategy, for instance informing the use of *in vitro* NAMs, or else may facilitate other *in silico* approaches such as read-across.

### 1.11.P-Th069 MechoA+: use, versatility and structural diversity

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*In silico* and computational approaches offer an efficient option for supporting safety decision making of chemicals. Mode and/or Mechanism of Action (MoA/ MechoA) profilers linked to robust quantitative structure-activity relationships (QSARs) and quantitative structure-property relationships (QSPR), provide valuable opportunities to reduce the need for *in vivo* studies. Last year one such tool, **MechoA+** was introduced and **MechoA Premium** (based on MechoA+ but with further additions and refinements) was implemented in iSafeRat® Desktop. This tool is a structure activity relationship (SAR) model developed to detect the molecular initiating events (MIE), the first step in the adverse outcome pathway by which substances induce adverse effects and some subsequent key events for a wide domain of species. Knowledge of the mechanisms is key to understand, predict and limit the impact of chemicals on humans and ecosystems. When entering the SMILES of substances in the tool, it detects if the substance potentially has a structural pattern known to be related to a given hazardous MIE and species. Using this knowledge, this SAR model has shown to have very versatile uses from product development stages to final steps of substances registrations. As such, it can:

- Raise alerts for undesired toxic mechanisms during the product development stage;
- Ensure mechanistic similarity in Read-Across approaches to fill regulatory dossiers data gaps;
- Select appropriate QSAR models to reliably predict (eco)toxicological outcomes;
- Rationalise toxicological effects observed in experiments.

Case studies of the use of MechoA+ in one or the other of these cases will be presented in the poster, e.g. Read-Across study, uses of the batch mode for Safe & Sustainable by Design (SSbD) process.

In this presentation, the structural applicability domain of the scheme will be challenged with a large variety of organic chemicals (e.g. common organics, biocides, plant protection products and pharmaceuticals) to demonstrate how much MechoA+ can cover. The results obtained will be presented.

### **1.11.P-Th070 How Well QSAR(s) Predict Aquatic Toxicity of REACH Registered Substances?**

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Information on aquatic toxicity is required under REACH Annex VII, Section 9.1.1 (Short-term toxicity testing on aquatic invertebrates) and 9.1.2 (Growth inhibition study on aquatic plants), Annex VIII, Section 9.1.3 (Short-term toxicity testing on fish), Annex IX, Sections 9.1.5 and 9.1.6 (Long-term toxicity testing on aquatic invertebrates and fish) [1]. All these standard information requirements can be adapted by a reliable and relevant QSAR adaptation (Annex XI, Section 1.3). The computational methods such as QSARs may not always carry information on all the effects measured in a e.g. OECD TG 210 study (hatching and survival, abnormal appearance, abnormal behaviour, weight, length). The purpose of this study was to assess if the use of QSARs would estimate hazards differently to standard experimental studies and whether any differences would affect regulatory decision-making. We collected aquatic toxicity data which were conducted after a Compliance Check (CCH) and Testing Proposal (TP) processes, and was accepted as being reliable in these processes. We predicted aquatic toxicity (EC50, EC10/NOEC) by QSAR models ECOSAR and VEGA for the substances for which new data was received. The experimental values were compared to the modelled predictions. At the end, we checked if there are substances that are predicted better or worse than the average. Moreover, we assessed if any deviation between experimental data and prediction would bring any effect in regulatory risk management by exceeding the specific thresholds (e.g., CLP classification criteria [2]). Initial results show that the predictions are in line with experimental data for some substances but for some, the prediction may differ significantly from the experimental effect concentrations. For some substances the differences between prediction and experimental data may also have an impact on regulatory risk management.

#### References

[1] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

[2] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures

### **1.11.P-Th071 Predicting species sensitivities to corticosteroids**

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Both biodiversity loss and concentrations of novel entities have exceeded planetary boundaries for future human generations to thrive. A key goal for humanity is to establish the link between these two –planetary boundaries and identify those chemicals of greatest concern and the species most vulnerable to reduce the impact on biota. This is a daunting task with a reported 350,000 synthetic chemicals produced globally and billions of existing species. However, the advancement in High Performance Computing (HPC) capacity, accessibility of genome and chemical databases and performance of high throughput in vitro screenings, offers an opportunity to discriminate differences at species level in molecular initiating events (MIE) leading to adverse outcomes. We have used the fish corticosteroid system as an exemplar of such an approach. We have shown that using protein structure prediction tools such as AlphaFold, and ligand binding docking programmes such as Kdeep, there are distinct difference in the predicted binding affinities (pKd) and Gibb free-energy of ligand binding (deltaG) between glucocorticoid receptor 1 and 2 and the mineralocorticoid receptor from over 50 species in response to both natural and synthetic corticosteroids. Furthermore, using an in vitro transactivation assay we have demonstrated that the EC50 values for gene transactivation correlates to the predicted pKd and deltaG values providing support to the use of computational methods as predictive tools for species sensitivities to environmental corticosteroids.

### **1.11.P-Th072 Can Fish Toxicity Data Reliably Predict Toxicity to Aquatic Stages of Amphibia – Re-evaluation of a Dataset by Formulation**

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A future risk assessment for amphibia will have to cover acute and chronic risk to aquatic and terrestrial stages. There are publications indicating that fish and tadpoles react similar to toxicants, but also papers claiming that amphibia might be more sensitive than fish. Toxic endpoints of both organism groups are listed in Appendix K of the supporting EFSA document (Ortiz-Santaliestra et al. 2017). Based on this dataset, the EFSA Panel proposed to apply an additional assessment factor (AF) of “at least 100” if acute fish-endpoints were used as a surrogate for acute amphibia endpoints. The dataset in Appendix K can however be improved, if matching fish endpoints (of the very formulation used in the amphibian test) are paired. These acute fish endpoints are available for virtually every formulation as a fish LC50 is a core data requirement. When updating the database accordingly, only few cases remained with fish less sensitive than amphibia, none by a large margin, and only these

would justify a high additional AF to bridge between fish and amphibian LC50-endpoints. If an additional AF of 100 were included in any future Guidance Document on top of the AF of 100 for acute fish endpoints, amphibian endpoints would have to be a factor of 10000 higher than the matching PEC. This would result in a very conservative risk assessment, so trigger many additional experiments with amphibia. Contradicting current efforts to reduce animal testing, EFSA even proposed to generate more experimental data with amphibia, until there was more evidence available. However, based on the revision of the existing data by formulation we conclude that there is already extensive evidence, indicating that acute risk to amphibians can be reliably predicted from fish endpoints, and hence additional acute animal tests are considered dispensable. An additional assessment factor of 100 to bridge between fish and aquatic amphibia stages should not be needed either. The data situation is similar to the one between cold-water- and warm-water-fish, the latter are no longer tested, as generally - though not always - less sensitive than cold-water fish, and no additional assessment factor was introduced for them. Also aquatic amphibia are generally less sensitive than (cold-water) fish, so no regular testing should be needed, neither an additional assessment factor.

#### **1.11.P-Th073 In vitro effects of plastic additives and their mixtures with pharmaceuticals on fish liver cell line**

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Wastewater treatment plants (WWTPs) effluents have been identified as a major route for release a large variety of contaminants into waterbodies particularly contaminants of emerging concern such as pharmaceuticals (PhACs) and microplastics (MPs). MPs in the marine environment can provoke direct physical effects, and act as dispersal vector for plastic additives, such as UV-filters or antimicrobials, that can be leached to the surrounding environment during physical disintegration processes. It can induce adverse biological effects, including synergistic or antagonistic interactions that may occur when organisms are exposed to mixtures of compounds with different modes of action. The main interaction between chemicals and organisms takes place at cellular levels, therefore cell systems are used as suitable early tools for detection of chemical exposure and toxicity.

Considering the key role of the liver in detoxification mechanisms, fish hepatocytes are often chosen as *in vitro* model systems. The use of permanent cell lines have several advantages over the use of animals *in vivo* exposure, such as their easy culture, availability and reproducibility. One well established permanent fish cell line is PLHC-1, derived from topminnow (*Poeciliopsis lucida*) hepatocellular carcinoma, which have been successfully used to assess the cytotoxicity, genotoxicity and oxidative stress of xenobiotics.

The present work developed in PHARMASEA project aimed to assess cytotoxicity and ability to counteract oxidative stress (through reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) assays) in fish liver cells exposed to 4 plastics additives from different nature (octocrylene, triclosan-TCS, bisphenol S-BPS and acetyl tributyl citrate-ACETYL) and their mixtures with citalopram, bezafibrate and carazolol at environmentally relevant concentrations. In addition, the wound healing assay was carried out to observe cells ability to growth and migrate until new cell-to-cell contacts are established.

The results obtained showed that none of treatment affected the wound healing. The different PhACs-ACETYL and PhACs-BPS mixtures had synergistic behaviour respect to individual compounds, while PhACs-TCS mixtures had antagonistic behaviour respect to individual compounds. The decrease of MMP in the single and mixtures at low concentrations, supported the hypothesis that there was an environmental risk due to their presence in the possible mixtures.

#### **1.11.P-Th074 In vitro effects of pharmaceuticals at environmental relevant concentrations on fish liver cell line**

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Pharmaceuticals (PhACs) have been quantified in marine environmental matrices at low concentrations (ng/L) and their occurrence in marine organisms raises concerns about harmful effects. Chemical analyses should always be combined with biological measurements in order to evaluate environmental risks. Therefore, it is very important to evaluate both PhAC bioavailability, as well as possible biological adverse effects, including synergistic or antagonistic interactions that may occur when organisms are exposed to mixtures of compounds. The main interaction between chemicals and organisms takes place at cellular levels, therefore cell systems are used as suitable early tools for detection of chemical exposure and toxicity.

Considering the key role of the liver in detoxification mechanisms, fish hepatocytes are often chosen as *in vitro* model systems. The use of permanent cell lines have several advantages over the use of animals *in vivo* exposure, such as their easy culture, availability and reproducibility. One well established permanent fish cell line is PLHC-1, derived from topminnow (*Poeciliopsis lucida*) hepatocellular carcinoma. PLHC-1 liver cells have been successfully used to assess the cytotoxicity, genotoxicity and oxidative stress of xenobiotics.

The present work developed in PHARMASEA project aimed to assess cytotoxicity and ability to counteract oxidative stress (through reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) assays) in fish liver cells exposed to the antidepressant citalopram, the lipid regulator bezafibrate, and the cardiovascular drug carazolol, and their mixtures at environmentally relevant concentrations. In addition, the wound healing assay was carried out to observe cells ability to grow and migrate until new cell-to-cell contacts are established.

The results showed that none of treatment affected the wound healing. As single compound exposure, carazolol didn't provide significantly different results compared to the control in the selected assays. Bezafibrate and citalopram induced the ROS production at low doses. Citalopram was the PhAC that most affected the normal functioning of cells. The different PhACs mixtures intensified the responses at lower concentrations. However, there were no differences in the responses obtained between tested concentrations in the mixtures. The decrease of MMP in the tertiary mix suggest that there is an environmental risk of PhACs due to their presence as mixtures.

### **1.11.P-Th075 Quantitative Adverse Outcome Pathway Assisted Formulation of Integrated In vitro Testing Strategies for Identification of Mitochondrial Uncouplers**

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There is an urgent demand for cost-effective new approach methodologies (NAMs) that fully align with the 3R principles (Replacement, Reduction, and Refinement), crucial for advancing current chemical risk assessment and regulatory processes. This case study aligns the AOP concept with the 3Rs, employing NAMs alongside a quantitative AOP network (qAOPN) to assess environmentally relevant uncouplers. Given the diverse impact of chemicals on mitochondrial functions, uncoupling of oxidative phosphorylation (OXPHOS) is a common mode of action for mitochondria toxicants, raising regulatory concerns such as growth inhibition and reproductive failure. This study demonstrates how integrating NAMs with qAOPN enhances the testing strategy, improving the cost-efficient hazard assessment of mitochondrial uncouplers. A qAOPN, using piecewise structural equation modeling (PSEM), was developed by quantifying key events (KEs) temporally in the zebrafish liver (ZF-L) cell-line. *In vitro* data, guided by AOP #263 & 264, utilized the model uncoupler carbonyl cyanide m-chlorophenyl hydrazone (CCCP). Validation involved a 96-hour fish embryo test and a 28-day fish early life stage test using zebrafish with overlapping CCCP concentrations. Measured KEs included OXPHOS, ATP level, cell death (metabolic activity, cell membrane integrity), and cell proliferation (CP). ZF-L was subsequently exposed to five environmentally relevant uncouplers (Perfluorooctanesulfonamide (PFOSA); 2,4-dinitrophenol (2,4-DNP); 2-(tert-butyl)-4,6-dinitrophenol (DTB); Chlorfenapyr (CFP) and Triclosan (TCC)) at  $1 \times 10^{-3}$  -  $3 \times 10^{-7}$  M. Temporal and concentration-dependent responses of ZF-L cells to CCCP were observed for KEs. Direct *in vitro* to *in vivo* comparison was challenging due to differing effect concentration by over one order of magnitude. PSEM generated 105 models, the most suitable testing strategy was ranked No. 1 and reported temporally regulated KE: OXPHOS 2h, ATP 6h, CP 24h, and Cell death at 24h, exhibiting strong predictability. Using this strategy, the study assessed the five uncouplers, revealing decreased mitochondrial potential, ATP, cell proliferation, and viability. The data will challenge the CCCP-based qAOP model, evaluating its predictive power for later KEs (CP and cell death) with environmental uncouplers. The study highlights the effective combination of (q)AOP and *in vitro* NAMs in screening mitochondrial uncouplers, offers novel causal knowledge and a workflow for qAOPN construction.

### **1.11.P-Th076 Transcriptomic Points of Departure in Early-life Stage Rainbow Trout Exposed to Diverse Chemicals for 24 hr**

*Emily Boulanger, Hugo Marchand, Kritika Mittal, Jessica Head and Niladri Basu, McGill University, Canada*

There is great interest in the development, validation, and application of New Approach Methods (NAMs) in the field of toxicity testing that are considered to be more cost-effective, ethical, and efficient. Transcriptomic points of departure (tPODs) are emerging as a promising approach for evaluating the toxicity of environmental contaminants while simultaneously obtaining mechanistic data. Here, the objective was to evaluate whether we could use a rapid 24 hr microplate-based embryo bioassay coupled with transcriptomics to assess the sensitivity of a chemical in rainbow trout (RT) alevins and generate tPODs. Briefly, individual RT alevins (1-2 day post-hatch) were placed into single wells (24-well plates) and exposed for 24 hr to a solvent control (fish water or 0.5% DMSO) and 11 concentrations of a chemical of interest (total of 12 individuals per concentration group). After the 24 hr exposure, alevins were pooled (n=3) to create 4 replicates per concentration group for differential gene expression and dose-response analyses. Presently, 25 chemicals have been tested using this microplate-based assay and we are currently in the process of generating tPODs for many of them. So far, RNA Sequencing (RNA-Seq) has been performed on RT samples for two chemicals of interest (i.e. ethinylestradiol (EE2) and 6PPD quinone (6PPD)). Significant genes ( $|\log_2FC| = 1$  and adjusted p-value  $< 0.05$ ) were fitted with a curve to determine the gene-level benchmark dose (BMD). From the distribution of all genes with BMDs, a tPOD was calculated on ExpressAnalyst. A transcriptomic point of departure was observed and tPODs were generated for EE2 (4.9 ng/L) and 6PPD (3.7 ng/L). The EE2 values are similar to a recent study in which RT hatchlings were exposed to EE2 from hatch to four days post hatch, following which RNA-Seq analysis of whole embryos yielded tPOD values ranging between 0.2 and 3.6 ng/L. Taken together, these findings will lend support to the notion that 24 hr microplate-based bioassays with RT alevins coupled with RNA-seq can yield molecular data



that can provide insights into mechanisms of action and are also conducive to benchmark dose modelling to derive quantitative tPODs.

### **1.11.P-Th077 Transcriptomic Points of Departure in Rainbow Trout Exposed to Ethinylestradiol: A Comparison of Approaches in Different Model Systems**

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To help move away from whole animal acute toxicity testing involving fish, there is interest in the development of alternatives to animal methods (e.g., early-life stage (ELS) and *in vitro* tests) that are high-throughput and can also derive quantitative transcriptomics point of departure (tPOD) values. To understand the variance in tPOD values across a range of experimental systems and conditions, here we examined different exposure models (cell line, embryo, juvenile) and transcriptomic methods (EcoToxChips, UPXome, RNA-sequencing) involving rainbow trout exposed to ethinylestradiol (EE2). In study #1, individual rainbow trout hatchlings alevins (1-2 day post-hatch) were placed into single wells (24-well plates) and exposed for 24 hr to a solvent control (0.5% DMSO) and 11 concentrations of EE2: 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 and 100 ng/L (12 individuals per group). After the 24 hr exposure, alevins were pooled (n=4) to obtain 3 replicates per dose group for gene expression analysis using the 384-gene Rainbow Trout version 0.1 EcoToxChip. Differential gene expression analysis and dose-response analysis methods were performed on EcoToxExplorer. In addition, RNA-Sequencing (RNA-Seq) was performed on the same set of samples and the data was analyzed using ExpressAnalyst. No mortalities or deformities were observed following EE2 exposure. Most differentially expressed genes (DEGs) from the EcoToxChip data were found in the 1 ng/L treatment group, and common DEGs included vitellogenin-like and estrogen receptor genes as would be expected with EE2 exposure. A tPOD was derived from EcoToxChips (4.86 ng/L) and RNA-Seq (4.80 ng/L) data. These values are similar to recently published studies in which rainbow trout: a) hatchlings (study #2; Alcaraz et al.) were exposed to EE2 from hatch to four days post hatch, following which RNA-Seq analysis of whole embryos yielded tPOD values ranging between 0.2 and 3.6 ng/L; and b) juveniles (study #3; Pagé-Larivière et al.) were exposed to EE2 from fry (60 days post-fertilization) for 76 days, following which microarray analysis of gonadal tissue yielded a tPOD value of 3 ng/L. Study #4 is underway with the rainbow trout gill cell line (RT gill-W1) exposed to similar concentrations of EE2 for 24 hr. Taken together, the findings from this case study of rainbow trout and EE2 lend support to the notion that tPODs generated from alternative to animal models can reflect tPODs from more involved animal studies.

### **1.11.P-Th078 Exploiting Single Cell Sequencing to Evaluate the Efficacy of Primary Gill Cell Cultures as Tools for Deriving Pharmaceutical Toxicological Mechanisms**

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New *in vitro* and genomic approaches are increasingly being incorporated into ecotoxicology. Such advances can be utilised to address novel challenges posed by emerging pollutants, in a way which is both representative and in line with the principles of the 3Rs. Even at environmentally relevant concentrations, some pharmaceuticals can elicit transcriptomic changes which can result in adverse outcomes. As vertebrates, fish share more drug target orthologues with humans than any other aquatic life and are at the most risk from this pollution. Repeated alterations to gene expression may cause chronic health issues, that are not necessarily detected by traditional acute toxicological assessment methods. Furthermore, these changes in the mRNA abundance can have a disproportionate impact on different cell populations. Thus, sensitive approaches are required to identify cell-specific molecular initiating events (MIEs) which drive subsequent adverse outcomes. Single-cell RNA sequencing (scRNA-Seq) is a promising technique for defining the precise mechanisms underpinning toxicity in individual cells. However, commonly used fish cell cultures are immortalised cell lines made up of one cell type, which can be genetically dissimilar to the original tissue. Alternatively, *Oncorhynchus mykiss* (rainbow trout) double-seeded gill inserts are a three-dimensional primary cell culture composed of different cell types, including small molecule transporters. These properties are why this culture is often utilised to study pharmaceutical toxicokinetics at the point of xenobiotic uptake. However, for this culture to also be used to study toxicodynamics through the lens of transcriptomic changes, it must first be demonstrated that mRNA profiles of the different cell populations are comparable to that of the original tissue. In this work, we aim to use scRNA-Seq to analyse the transcriptomic fingerprint in both the double-seeded gill model and the original gill tissue to identify if there are significant differences in cell type abundances and transcriptomes. If this model proves to be sufficiently representative, further scRNA-Seq could be conducted to elucidate the subtle low-dose effects that pharmaceuticals are having on specific cell populations, without the need for *in vivo* study. Employing scRNA-Seq in aquatic ecotoxicology can improve the mechanistic understanding of toxic effects, in a way that is highly suitable for deriving the mode of action of pharmaceutical pollutants.

### **1.11.P-Th079 Exploring the genome of the oribatid mite, *Oppia nitens***

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Oribatid mites are one of the most abundant group of microarthropods in soil. *Oppia nitens*, belonging to the family Oppiidae, one of the largest and diverse families of oribatid mites, has been developed as a standardized model test organism for the assessment of soil contamination and habitat adaptation. However, the limited availability of genomic information for this species hinders our understanding of its physiological adaptation and sensitivity to chemical stress and soil habitat quality. We

present the annotated *O. nitens* draft genome assembled using both Oxford Nanopore Technologies and Illumina sequencing platforms.

The sequences assembled into 65 scaffolds spanning 125.4Mb with a 24.5% GC content, a N50 length of 4.41Mb, and a maximum scaffold length of 6.36 Mb. Genome quality and completeness were checked using arthropod Benchmarking Universal Single Copy Orthologs (BUSCO) analysis, which identified: 96.9 % - 93.5 % complete single-copy orthologs, 3.4% complete but duplicated orthologs, 0.5% fragmented, and 2.6% missing orthologs (n=2934). The mitochondrial genome was also recovered and assembled. We found 15,369 protein-coding genes, 16, 969 mRNA, and 14, 938 proteins.

Here, we describe the *O. nitens* reference genome and discuss its utility as a genetic basis for further investigations and understanding of the molecular mechanisms and physiological functions in adaptations to environmental change, especially exhibited tolerance of *O. nitens* to metal stress.

### **1.11.P-Th080 Sub-lethal Transcriptomic Points of Departure and Toxicity Pathways of Legacy and Emerging Perfluoroalkyl Substances Determined Using New Approach Methods**

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Per- and poly-fluoroalkyl substances (PFAS) have unique, amphiphilic properties that extend them to many uses in industry and commercial products. As a result, some PFAS have become ubiquitous in the environment. Recently, several 'replacement' PFAS have been produced and are beginning to emerge in the environment. Two such emerging compounds, perfluorobutane sulphamide (FBSA) and perfluoroethylcyclohexane sulphonate (PFECHS) are becoming widely detected, however, little to no information regarding their effects at either the molecular or apical levels is available. Therefore, this study investigated the toxic potency and mechanisms of toxicity of these substances at sub-lethal effect concentrations related to perfluorooctane sulphonate (PFOS). Zebrafish (*Danio rerio*) embryos were chosen as the experimental model to investigate three apical endpoints: (1) mortality, (2) abnormalities, and (3) growth, as well as to be sequenced using mRNAseq to obtain transcriptomic datasets. The embryos were exposed from 24 to 96 hours post fertilization (hpf) to seven exposure concentrations from 0.0001 – 5 mg/L plus a solvent control of each compound individually, with a subset extracted for total RNA, which was then sent to Genome Quebec Inc. for RNA sequencing. Each compound resulted in a maximum mortality rate < 20%, with the order of compound potency being PFOS > PFECHS > FSBA. Focusing on the highest treatment versus the solvent control, the PFOS exposure resulted in 539 differentially expressed genes (DEGs), the PFECHS exposure resulted in 24 DEGs, and the FSBA exposure resulted in 630 DEGs. However, only 497, 6, and 2 genes followed dose-response relationships for PFOS, PFECHS, and FSBA, respectively. The low amount of DEGs recorded for PFECHS and FSBA can likely be associated with the relatively low apical toxicity observed under these two compounds. As a result, only tPoDs for PFOS could be determined and gene level benchmark doses (BMDs) were calculated as 0.00012, 0.00016, and 8.7e-5 mg/L for the 20<sup>th</sup> gene, max 1<sup>st</sup> peak, 10<sup>th</sup> percentile BMDs, respectively. The DEGs for PFECHS and FSBA were related to adrenergic signalling in cardiomyocytes, suggesting this pathway may be driving the enrichment signal for the gene sets identified. Determining the toxic potency of emerging replacement PFAS is an important step that will help better inform the viability of replacements as a strategy for PFAS management in the future.

### **1.11.P-Th081 The Induction of Nuclear Receptor Pathways Plays a Role in Hepatic Thyroid Hormone Metabolism in Zebrafish (Danio Rerio) Embryos**

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Conjugation of thyroid hormones (TH) as biotransformation phase II metabolization can be divided into two major pathways: Glucuronidation by UDP-glucuronosyltransferases (UGTs) and sulfation by sulfotransferases (SULTs). These reactions primarily increase water solubility and thus facilitate biliary excretion of THs. While disruption *via* hepatic induction as part of an adverse outcome pathway (AOP) has been observed in multiple mammalian species, it has thus far not been explored in detail in fish. The expression of these conjugation enzymes is influenced by a variety of nuclear receptors most commonly associated with the detection of xenobiotics. Examples include the aryl hydrocarbon receptor and the pregnane x receptor. These accept a wide range of potential agonists, and their activation generally leads to an increase in the expression of enzymes involved in the metabolization of xenobiotics. As an off-target effect, UGT and SULT expression can also be increased to levels that negatively affect TH homeostasis.

In the context of the 3R principles, it would be highly beneficial to determine whether fish and other lower vertebrates are also sensitive to TH system disruption *via* this mechanism. Specifically, the applicability of zebrafish (*Danio rerio*) embryos as a predictive model would help to reduce mammalian animal testing. First exposure experiments provided strong indication towards this being the case, showing effects of hepatic inductors on the morphological level. To appropriately investigate this mechanism, further experiments are necessary to properly evaluate the induction of hepatic TH metabolism in zebrafish. These range from tests on the molecular level to the assessment of possible behavioural changes resulting from altered TH signalling. For this end, wildtype zebrafish were exposed to different known agonists of the nuclear receptors involved in UGT and SULT expression to cover different molecular key events. These experiments assessed endpoints related to molecular events and possible behavioural changes resulting from altered TH signalling and involve endpoints like TH levels, eye histopathology,

swim bladder inflation and visual motor response. The experiments aim to deepen the understanding of hepatic TH metabolism in zebrafish embryos. Comparisons with data from other vertebrates will reveal similarities and differences among species and vertebrate classes.

### **1.11.P-Th082 Navigating truth and truce: Validation as institutional entrepreneurship in regulatory science**

*H.M. Tuihedur Rahman<sup>1</sup>, Gordon Hickey<sup>1</sup> and Steven Maguire<sup>2</sup>, (1)McGill University, Canada, (2)University of Sydney, Australia*

Regulators responsible for national and international chemicals management need New Approach Methodologies (NAMs) to overcome the ethical and technical limitations of prevailing animal-based risk assessment approaches. Validation – a process based on sound scientific principles to establish the reliability and relevance of a test method (OECD, 2005) – is an important step towards regulatory acceptance and adoption of NAMs (and NAM-generated data) in chemicals management decision making. However, risk assessment of chemicals is a highly institutionalized field of professional practice, with the methods, routines and tools used by risk assessors – and widely accepted by regulators and industry actors – largely taken for granted, even if practitioners recognize their shortcomings. As a result, these entrenched practices are difficult to change, and doing so requires “institutional entrepreneurship” – “activities of actors who have an interest in particular institutional arrangements and who leverage resources to create new institutions or to transform existing ones” (Maguire et al., 2004: 657). This presentation draws on a case study of the entrepreneurial activities of members of a NAM innovation project team – referred to as NAMTEAM – which aims to develop a low-cost, and reliable toxicogenomic technology. We collected qualitative data from the project’s initial proposals, monthly updates, recorded yearly meetings, validation plan, publications, workshop reports, and other correspondences related to validation activities. Our analysis reveals that regulatory actors’ understandings about validation are diverse but can be broadly grouped under two ideal types in tension: a “scientific logic” in which validation signifies ‘truth’ vs a “political logic” in which validation signifies an end of contestation and social acceptance or ‘truce’. Institutional entrepreneurs navigate this tension in a variety of ways. Since validation activities are not value neutral and may change with scientific advances, validation plans and activities need to be reflexive and adaptive. The findings of this study can inform multi-level efforts to advance the use of NAMs in regulatory science and risk assessment processes.

### **1.12 Omics Beyond Transcriptomics: Leveraging Proteomics and Metabolomics to Improve Mechanistic Understanding of Responses to Environmental Stressors**

#### **1.12.T-01 Metabolomics response kinetics of a periphytic community to mixture of pesticides to unravel short term molecular mechanisms involved in long term structural and functional impairment**

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Agriculture leads to the release of multiple pesticides into aquatic ecosystems that might be harmful for exposed organisms such as periphytic microbial communities playing a key role in ecosystem functions. Despite evidence about the effect of these chemicals on the structure and functions of periphytic biofilms, there is still a paucity of knowledge about the kinetic of responses. In particular, there is a need to unravel how short term molecular responses are involved in long-term impairment at the functional/structural levels to support the discovery of biomarkers allowing the early and sensitive detection of long term alteration of ecosystem function(s). To this end, untargeted meta-metabolomics is a cutting edge approach since it provides a comprehensive picture of the molecular phenotype of the whole community, as a result of interactions with the environment. In this context, this study aims to characterize the link between the response at the molecular level and the responses at the physiological/function and structural levels of periphytic biofilms exposed during one month to mixture of insecticide (fenoxycarb,FNX), herbicide (glyphosate, GLY) and its metabolite (AMPA). At the structural level, normal increase of the biomass was observed between 13 and 28 days in control and FNX conditions while the growth was lower in condition with GLY+AMPA and the mixture. In parallel, there is a decrease of cyanobacteria in control and FNX conditions while they increase following exposure to GLY+AMPA and the mixture. At the physiological level, significant discrepancies were noted for the photosynthetic yield with a decrease after 13 and 28 days of exposure to GLY+AMPA, FNX and the ternary mixture. At the molecular level, HCA showed a clustering of the features according the time as soon as after 4h of exposure. At this time, the effect of the conditions GLY+AMPA is already visible in comparison to other conditions. Further comparison of the significantly modulated features highlighted few overlap between the tested conditions suggesting that specific metabolic pathways were involved in the response. Altogether, our results highlight that the microbial community metabolome changed strongly over time and also responded very quickly to pesticides exposure. This study also suggests specific metabolic pathways involved in the response to FNX vs GLY+AMPA vs FNX+GLY+AMPA, and so likely specific adverse outcome pathways leading to ecological functions impairment.

#### **1.12.T-03 Xenometabolome of Early Life Stage Rainbow Trout Exposed to 6PPD-quinone**

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPDq) is toxic to several salmonid species. The differences in metabolism of this compound in fishes is an emerging area of research, as it leads to detoxification or potential activation of the analyte. Mass spectrometry can prioritize metabolites of concern while using data-dependent acquisition (DDA) for suspect

screening novel metabolites and understanding potential impacts on the endogenous metabolome. This study used targeted and untargeted metabolomics to determine the metabolism of 6PPDq in larval rainbow trout and assessed the effects of 6PPDq exposure on the metabolome. Larval rainbow trout tissue was extracted for targeted 6PPDq metabolite analysis using a full MS/parallel reaction monitoring method with analyte separation using Vanquish UHPLC and positive scans using QETM HF Quadrupole-Orbitrap™ mass spectrometer with HESI ion source (Thermo Scientific). Untargeted analysis utilized a DDA to acquire ddMS2 top 5 data, and the resulting DDA data was analyzed using mzMine3 for spectral preprocessing and feature detection before exporting to GNPS for feature-based molecular networking (FBMN) and to SIRIUS for molecular formula prediction and annotation. Greater levels of hydroxylated 6PPDq were detected in larval fish tissue compared to 6PPDq glucuronide conjugate. Metabolites of 6PPDq detected via FBMN and Cytoscape were found to be associated with the precursor 299.1746 m/z (6PPDq) for hydroxylation transformation to form an alkyl hydroxylation transformation product (m/z = 315.169), aromatic ring hydroxylation, and a double hydroxylation (m/z = 331.1644). Another network detected for phase II biotransformation of 6PPDq included glucuronidation of single hydroxylation (m/z = 491.2014) and double hydroxylation (m/z = 507.1965). An additional phase II transformation product was detected for sulfonation of single hydroxylation (m/z = 395.1265). PLS-DA revealed the separation of the concentrations in multivariate space, and several metabolites were important in driving these differences in a dose-response manner. The next steps include further identification of dysregulated metabolites in larval rainbow trout and characterization of the metabolism of 6PPDq in larval fathead minnow, brook trout, and brown trout, allowing for comparison of the metabolism of 6PPDq in greater phylogenetic space and in nonsensitive species.

#### **1.12.T-04 Phosphoproteomics-Based Investigation of the mTOR Pathway Signalling Reveals its Role in Mediation of Chemical Effects on Fish Cell Culture Growth**

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Many signalling cascades in the cells are regulated by (de)phosphorylation at specific protein sites. Several initiator kinases can respond to diverse stressors, including chemical exposure. However, mechanistic exploration of chemical effects on protein phosphorylation has received little attention in predictive (eco)toxicology so far, likely due to technical limitations. For example, in non-mammalian model organisms, suitable antibodies are often lacking. To address this limitation, we worked to develop a mass spectrometry-based targeted (phospho)proteomics method, using the zebrafish (*Danio rerio*) PAC2 cell line model and focusing on the mTOR kinase pathway, which is known to regulate growth and could also play a role in mediating chemical effects on growth. The optimized workflow includes fast cell lysis with 5% SDS, S-Trap™ protein isolation and digestion, followed by NTA-Fe phosphopeptide enrichment. Currently, abundance and/or phosphorylation status can be monitored for 21 protein targets within the mTOR pathway simultaneously. We used this method to study the mTOR pathway responses to pharmacological inhibition of the mTOR kinase by Torin2 as well as nutrient deprivation stress and exposures to chemicals known to inhibit fish growth. As an example, Torin2 showed no acute toxicity but caused a strong reduction in cell culture growth in chronic scenario. Protein abundance was affected only slightly, while protein phosphorylation changes in response to exposure were more dynamic and suggested impacts on several upstream regulators of the mTOR pathway activity. Ongoing study investigates whether early responses on the (phospho)protein level can be used to predict reduced growth as measured at a later time point, through gaining a time-resolved view into mTOR pathway dynamics in response to chemicals known to inhibit fish cell culture growth and/or fish growth *in vivo*. Collectively, our results suggest that the mTOR in fish plays a similar role in the regulation of cellular growth and proliferation as that known in mammals. In a broader context, this study demonstrates the applicability of the mass spectrometry-based targeted (phospho)proteomics as a feasible and versatile approach to study chemical disruption of phosphorylation-based signalling in different organisms. Better understanding of the roles played by signalling pathways could allow identifying novel mechanistic targets and exploring their suitability as predictive markers of toxicity.

#### **1.12.P Omics Beyond Transcriptomics: Leveraging Proteomics and Metabolomics to Improve Mechanistic Understanding of Responses to Environmental Stressors**

##### **1.12.P-We077 Is Toxicophenomics The New Omics?**

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Risk assessment (RA) aims to characterize the response of organisms to a stressor, which can be achieved at different levels, through different methods, and with different data types. However, not all levels, methods, and data types have received the same attention. Within the omics field, this is the case for phenomic data. Phenomics, defined as the “acquisition of high-dimensional phenotypic data on an organism-wide scale”, has great potential for predicting important biological outcomes, but it is not fully exploited as technological advances have been limited. In the context of environmental stressors and toxicological research, toxicophenomics are of great interest, as it combines phenomics and toxicology. However, the limited advances in phenomics also create a knowledge gap in toxicophenomics.

This study aims to extend the knowledge of toxicophenomic data, its opportunities, and its challenges. We hypothesize that toxicophenomic data will convey extra knowledge and deepen our understanding of responses to environmental stressors. This knowledge will be useful to improve RA.

The toxicophenomics data used for this study originates from a dose-response experiment where seven non-target plant species were exposed to glyphosate. The plants were monitored in a fully automated high-throughput phenotyping platform. Non-destructive measurements were performed repeatedly during the experiment and destructive measurements were obtained at the end of the experiment.

The data will be analyzed in R using the “drc” and “bmd” packages for fitting dose-response models and extracting benchmark doses. The analysis will shed light on the information that can be extracted from such data. As an example, the porometer measurements, such as stomatal conductance, can provide insights on the efficiency of photochemistry, and stress recovery. Data from thermal imaging can provide information on responses to biotic and abiotic stress, for example, to detect diseases. Multispectral imaging can be used to quantify growth rates and study early responses to drought stress. The chlorophyll content provides insights on photosynthesis efficiency, while the anthocyanin and flavonol contents can be used to study response to stress, e.g. drought resistance.

This study will shed light on the unique knowledge that can be acquired through toxicophenomic data. This will enable the integration of toxicophenomics in RA procedures and will provide novel knowledge that can improve RA.

### **1.12.P-We078 Coupling Uncoupling: Deciphering the Molecular Symphony – Exploring the Impact of Mitochondria Uncoupling Chemicals on the Transcriptome and Metabolome of Zebrafish Embryos**

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Mitochondrial uncoupling chemicals manifest their effects by disrupting oxidative phosphorylation (OXPHOS), a main metabolic pathway that produces ATP. Uncoupling of OXPHOS can cause reduced ATP production and cell proliferation and adverse effects of regulatory concern, such as growth inhibition. Uncoupling of OXPHOS leading to growth inhibition (AOPWiki, AOP #263) has been proposed as a key adverse outcome pathway (AOP) in eukaryotes and endorsed by the Organisation for Economic Cooperation and Development for potential regulatory use. This study aims to utilise new approach methodologies (NAMs), in particular the zebrafish embryo model as an alternative to fish testing, to generate in-depth systems biology and quantitative understanding to support AOP #263, develop and standardize new NAM testing strategies and expand this AOP to a more complex AOP network (AOPN). Zebrafish embryos were exposed to different concentrations of the reference mitochondrial uncoupler carbonyl cyanide m-chlorophenyl hydrazone (CCCP) from 3 to 96 hours post fertilisation (hpf). Targeted bioassays (uncoupling of OXPHOS, ATP pool, cell proliferation and growth), histopathological analysis and a regulatory toxicity protocol (i.e., Fish Embryo Toxicity Test, OECD TG 236), multi-OMICS (transcriptomics and metabolomics) as a high-content NAM was used to generate new knowledge on the temporal and concentration-dependent stress response patterns in response to mitochondrial uncoupling. Furthermore, a multi-OMICS point of departure (POD) estimation using benchmark dose (BMD) modelling will be performed to support the AOP and identify new key events (KEs) in a larger AOPN. ATP pool and growth were affected in a concentration-dependent manner. Significant effects on ATP pool were observed in 48hpf and 72hpf embryos. Significant growth inhibition was observed in 96 hpf embryos. OMICS analysis revealed a high number of commonly affected genes and metabolites across concentrations and time. Pathways such as ECM-receptor interaction, retinol metabolism and apoptosis are found to be highly enriched. In the next step, BMD modelling and subsequent functional enrichment analysis will be performed to support AOP #263 in terms of temporal and dose concordance. This study uses combined multi-OMICS and phenotypic bioassays, as NAMs to generate systems toxicological data in zebrafish embryo exposed to a model mitochondrial uncoupler and the results will be used to expand the current AOP to an AOPN.

### **1.12.P-We079 Metabolomic changes in juvenile coho and Chinook salmon exposed to the vehicle tire associated chemical 6PPD-quinone**

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*N*-(1,3-Dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone, 6PPD-Q), an ozonation product of the tire rubber antioxidant 6PPD, has been identified as an important driver of urban runoff mortality syndrome. This phenomenon affects adult coho salmon (*Oncorhynchus kisutch*) upon entry into urban streams and results in high rates of pre-spawn mortality. Studies have reported a wide range of sensitivity to 6PPD-Q in several fish species. Our recent study revealed more than 3 orders of magnitude difference in the LC50 values between newly feeding juvenile Chinook (*Oncorhynchus tshawytscha*) and coho, with coho being the most sensitive. Both etiology and sublethal effects of 6PPD-Q are not well understood, although capillary leak and disruptions of the blood brain barrier have been associated with 6PDD-Q induced coho mortality. Here we investigated the sublethal effects of 6PPD-Q in newly feeding juvenile coho and Chinook salmon using a targeted metabolomic platform in a subset of samples collected during our recent acute 24h exposure. Sample exposure concentrations ranged from 11.9-38.5 ng/L for coho, and 3 112-25 808 ng/L for Chinook. Up to 530 and 556 metabolites of the 633 targeted panel were detected in juvenile coho and Chinook, respectively. Our preliminary results reveal altered metabolite profiles in both species in response to 6PPD-quinone concentrations, which differ between the two species, and may be

consistent with their varied sensitivities. Ongoing metabolomics data analysis in the two salmonid species will identify the main metabolic pathways involved in the sub-lethal responses to 6PPD-Q exposure.

#### **1.12.P-We080 Omics-Based Biomarker Selection for Difenoconazole and Metalaxyl Toxicity in Zebrafish Embryos.**

**Fatma Marghany<sup>1</sup>, Steve Uwa Ayobahan Dr.<sup>1</sup>, Gabriela Salinas<sup>2</sup>, Christoph Schaefers<sup>3</sup>, Henner Hollert<sup>4,5</sup> and Sebastian Eilebrecht<sup>1</sup>, (1)Ecotoxicogenomics, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (2)NGS - Integrative Genomics Core Unit, Germany, (3)Ecotoxicology, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (4)Environmental Media Related Ecotoxicology, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (5)Goethe University Frankfurt, Evolutionary Ecology and Environmental Toxicology, Germany**

Due to increasing application of fungicides in agricultural, veterinary and medicinal practices, relevant concentrations of fungicide residues in the environment as well as crops have been detected by previous studies. Beside the reported adverse effects of fungicides on different organisms inhabiting the ecosystem, such observations prioritise developing efficient and sensitive approaches for predicting environmental hazards of fungicides. In this study, we applied OMICs analysis to identify substance-specific biomarker candidates which may act as early indicators for ecotoxicity of difenoconazole (sterol biosynthesis inhibitor) and metalaxyl (nucleic acid metabolism inhibitor). Zebrafish embryos were exposed to the fungicides according to a modified protocol of the OECD test guideline No. 236, terminated by simultaneous RNA and protein extraction in order to analyse the whole transcriptome and proteome. Consequently, a positive exposure-response correlation was detected at differentially expressed gene (DEGs) and differentially expressed protein (DEPs) levels in all test concentrations for both fungicides. Moreover, a positive correlation between the obtained transcriptome and proteome responses was observed, denoting the persistence of the detected molecular responses. From the detected DEGs, candidate biomarkers specific for difenoconazole (*apoa1b*, *gatm*, *mylpfb* and *acta1b*) and metalaxyl (*lgals2b*, *abat*, *fabp1b.1* and *myh9a*) were selected, and their biological functions were discussed to assess the predictive potentiality of our approach. The developed biomarker panels may act as early indicators for environmental hazards of chemical substances under development. Furthermore, the integration of OMICs techniques in ecotoxicology can enhance our understanding of key events (KE) and key event relationships in an Adverse Outcome Pathways (AOPs).

Key words: Zebrafish Embryo, Fungicides, Transcriptomics, Proteomics, Biomarkers, RNA Seq

#### **1.12.P-We081 The Effects of Hypoxia on Fathead Minnow Behaviour and ‘Omics**

**Raina Hubley, Aisha Pasha, Meghan Allen, Theresa Warriner and Denina B.D. Simmons, Ontario Tech University, Canada**  
Behaviour is a useful parameter for monitoring the health of vertebrates in the context of environmental changes. Behavioural monitoring is non-invasive and could be utilized as a refinement for fish studies in both the lab and field environments. Traditional ‘omics methods can be utilized to determine changes in fish protein, metabolite, and lipid abundance due to toxic exposures. Dissolved oxygen fluctuations in water bodies have become more prevalent due to climate change and these fluctuations can lead to increased frequency and intensity of hypoxic conditions. We aimed to combine non-invasive methods with traditional methods to determine the effect of hypoxia on Fathead minnow (*Pimephales promelas*) behaviour and ‘omics. We exposed 60 Fathead minnows to hypoxic (2.10-2.80 mg/mL DO) and normoxic (5.80-7.00 mg/mL DO) conditions over a 7-day period. Fish were video recorded on days 1, 3, 5, and 7. Three blind observers analyzed the videos for fish tank location activity, foraging behaviour, and novel object behaviour. On day 8, we anaesthetized fish with 100 mg/L MS-222 and collected mucus, plasma, gill, and brain samples and also measured blood glucose. We found that hypoxia altered Fathead minnow activity levels over the course of the 7-day treatment. In this presentation, we will share the integrated results of fish behaviour, brain ‘omics, mucus ‘omics, and blood glucose levels

#### **1.12.P-We082 Non-targeted multi-omic analyses of blood plasma for health exploration of namew (lake sturgeon, *Acipenser fulvescens*) in an intact and an impacted watershed in the Moose Cree Homeland**

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Namew (nah-may-yo) or lake sturgeon (*Acipenser fulvescens*), are large, long-lived, benthivorous fish that are ecologically and culturally significant to many Indigenous communities. Historically, they were found throughout most of North America's Hudson Bay, St. Lawrence, and Mississippi drainage basins. Overexploitation and extensive habitat loss and alteration since the 1800s caused massive declines in Lake Sturgeon abundance over much of its range. The Moose Cree Homeland includes a diversity of rivers for namew, some of which are heavily impacted by hydroelectric development, forestry, mining, and other impacts, while others are free-flowing and without any development. Therefore, these internationally endangered fish face impacts and threats at varying levels of severity within the Moose Cree Homeland. The People of the Moose River Basin have compiled over a century of knowledge regarding the impacts of hydroelectric development and activity on Moose Cree Peoples, including declines in namew in some areas of the Moose Cree Homeland. Our collaborative team examined fine scale measures of namew health and condition in the Mattagami River, which has been impacted by hydropower, forestry, and other industrial development, and the free-flowing and unimpacted North French River, both tributaries of the Moose River system. We used non-targeted metabolomics, lipidomics, and proteomics to profile and investigate location-specific variation among detected biomolecules, before functionally characterizing biomolecules to identify possible health effects for namew in these

two river systems. We present our findings from one sampling season and discuss our future non-targeted multi-omic approaches.

### **1.12.P-We083 Untangling Defensomes: Omic Comparisons Across Species and Chemical Mode of Action to Aid Species Sensitivity Prediction.**

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Terrestrial invertebrate groups are declining, with pollution thought to be a major driving factor. Faced with the challenge of translating model species data into predictions for the wider range of species in ecosystems, risk assessment would be improved with accurate prediction of species sensitivity that incorporates a deeper molecular understanding of species-toxicant interactions. Current research characterising such molecular biology has (for good reasons) largely focussed on toxicant receptors (i.e. the initiation event that triggers downstream consequences) and cross-species comparisons of adverse outcome pathways. However, other factors (not adequately incorporated into existing approaches) can also dictate chemical toxicity to non-target organisms, such as including a species' capacity to mount an orchestrated response to exposure. This response (defensome) includes modulating the genes linked with chemical detoxification, elimination, damage mitigation and repair. The diverse evolutionary paths and life histories across species means defensomes will not be equal, with some species possessing more or less optimal responsive capacities to varied chemical and environmental stress. To leverage such defensomic data, major questions need to be answered for many invertebrate groups. For example, to what extent do defensome responses vary across chemicals within the same species, as well as across species for the same chemical. And, just as critically, to what extent do such differences explain (and therefore predict) sensitivity. We have characterised the transcriptomic responses of the annelid *Enchytraeus crypticus* across time (6 time points from 1hr-24hrs) exposed to equivalent sub-lethal doses of pesticides (EC<sub>50</sub> for reproduction) that act via varied modes of action. As well as revealing both the core (i.e. common to all chemicals) and pesticide-specific defensomes, we score defensomes in terms of differential inducibility and magnitude of response, correlating these scores with sensitivity. Furthermore, to compare defensome scores across species, we have sequenced the transcriptomes of larval *Mamestra brassicae* (Cabbage Moth) following sub-lethal exposure to a subset of the same pesticides. We discuss our findings in the context of understanding the contribution varied defensomic responses make to a species' sensitivity, as well as the challenge of defensome characterisation in species that are relatively poorly studied at the molecular level.

### **1.12.P-We084 Development of Proteomics Modules for the OECD Omics Reporting Framework**

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The OECD Omics Reporting Framework (OORF) was developed to facilitate the regulatory use of omics data from laboratory-based toxicology studies, which is achieved by means of (i) increasing transparency and reproducibility, and (ii) supporting standardization and harmonization. The latter can in turn improve the efficiency of data sharing and promote its use in systematic reviews and meta-analyses. The OORF consists of a narrative guidance and reporting templates that cover the study design and data generation, processing and analysis stages. Additional modules focusing on selected application cases, such as read-across, can also be included.

The first edition of OORF, released in November 2023 (ENV-CBC-MONO(2023)41.en), focused on transcriptomics and metabolomics studies, while proteomics was not covered due to less frequent use in the past. However, since technological capabilities for proteomics analysis have greatly improved recently, the number of studies providing protein-data based insights into toxicity mechanisms and effects is expected to increase rapidly. Therefore, in order to support the use of proteomics data for regulatory purposes, this project aims to develop respective proteomics modules for inclusion in the OORF.

Based on the initial scoping survey, we prioritized the development of two proteomics-relevant Data Acquisition & Processing Reporting Modules (DAPRMs), namely (1) mass spectrometry-based proteomics (including both global and targeted acquisition, as well as label-free and label-based quantification methods), and (2) affinity proteomics. Gel-based proteomics

were excluded from the scope, as this technique is rarely used for regulatory toxicology studies, if at all. In addition, the Data Analysis Reporting Modules (DARMS) currently present in the OORF will be reviewed and updated to enable their application for reporting proteomics data as well.

The inclusion of proteomics-specific modules in the OORF is expected to facilitate the maturation of proteomics-based toxicity assessment methods and allow regulatory consideration of proteomics data. This contributes to delivering on the original vision of building a comprehensive OORF that covers the major omics data types most frequently collected in chemical toxicity studies, thus enabling an efficient and unbiased use of these data streams in chemical hazard and risk assessments performed by regulators, industry and other stakeholders. *This abstract does not reflect US EPA policy.*

### **1.12.P-We085 Unravelling the Modes of Action of Antibacterial Agent Triclosan and Novel Alternatives in Human Macrophages by Untargeted Proteomics**

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Triclosan (TCS) is an antibacterial active agent, which regulatory agencies in Europe and the US restricted due to its diverse effects on human health, rendering substitution products more relevant. TCS influences the immune metabolism in macrophages by activating the NLRP3 inflammasome, but effects on the molecular level are not entirely understood and knowledge about the alternatives is rare. Hence, we aimed to unravel the potential adverse effects of TCS and its substitutes in human macrophages.

To decipher the underlying modes of action of the antimicrobials, endotoxin-stimulated THP-1 macrophages were treated with TCS or the alternatives, including chlorhexidine (CHX) benzalkonium chloride, benzethonium chloride, chloroxylenol and cetylpyridinium chloride. First, the cytokine release was analysed using ELISA, showing significantly higher TNF and IL-1 $\beta$  in TCS-treated cells than the control. In contrast, CHX completely abolished cytokine release. A reduction of TNF and IL-1 $\beta$  was determined among the other investigated alternatives, and IL-6 was reduced among all treatments.

Molecular changes were studied using untargeted LC-MS/MS-based proteomics applying tandem mass tags (TMT), and effects on the pathway level were determined using Ingenuity Pathway Analysis®. TCS and CHX showed the strongest response at the protein and signalling pathway levels after 24 h of treatment. Due to oxidative stress conditions, both agents inhibited signalling pathways related to metabolism, like glycolysis, TCA cycle and oxidative phosphorylation. While TCS was responsible for mitochondrial dysfunction resulting in inflammasome activation, CHX induces translational arrest. Therefore, the formation of stress granules was determined in CHX-treated cells using fluorescence microscopy. Besides, podosome formation was observed, potentially resulting in ECM degradation. Likewise, small and large ribosomal proteins and proteins related to the actin cytoskeleton were identified as putative key drivers by a networked-based analysis (WGCNA).

The obtained data showed significant alterations of the inflammatory response in macrophages by TCS and substitute exposure, especially CHX. The results illustrate the need to understand molecular mechanisms for developing strategies to minimise adverse effects.

### **1.12.P-We086 Acetylation and Phosphorylation are Dynamically Involved in Adipocyte Differentiation and Provide Insight on their Response to the Emerging Plastic Additive DINCH**

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Obesity is a major public health burden impacting life quality and expectancy of more than one billion people worldwide. Factors beyond genetic predisposition and modern sedentary lifestyle may facilitate the development or progression of obesity. Metabolism-disrupting chemicals (MDCs) ubiquitously present in our environment are external factors that can promote obesogenic onset by interfering with endocrine regulation yet causing metabolic conditions. The molecular mechanisms underlying the exposure to MDCs in adipose tissue are incompletely understood. Especially for emerging contaminants, fundamental knowledge about their modes of action is lacking. Proteins critically control biological processes, with their post-translational modifications (PTMs) serving as proxies of cellular signalling.

Hence, we analysed the PTMs acetylation (AcK) and phosphorylation (PP) during adipocyte development and during exposure to the emerging plastic additive DINCH (1,2-cyclohexanedioic acid diisononyl ester) and its active metabolite MINCH



(monoisononylcyclohexane-1,2-dicarboxylic acid ester), to gain insight into molecular events triggered upon exposure. We combined mass-spectrometry based proteomics with PTM enrichment strategies on human SGBS and murine 3T3-L1 adipocyte cell lines as well as visceral, subcutaneous, brown adipose tissue and liver of C57BL/6N mice following dietary exposure to DINCH for 16 weeks.

During healthy adipogenesis of SGBS and 3T3-L1 cells, we observed a dynamic involvement and distinct time-dependent profiles of the acetylome and phosphoproteome. AcK was dominantly involved in core metabolism while PP was found important for cell structural organization and insulin signalling, both with great relevance for the development of metabolic co-morbid conditions. MINCH exposure of SGBS adipocytes altered the PTM profiles of glycolysis/gluconeogenesis (AcK) and insulin signalling (PP) and pointed to the Rab effector WDR44 as relevant phosphorylation target. Dietary exposure to DINCH *in vivo* indicated no distinct phenotype, but appeared to be associated with distinctive liver damage, inflammation and disturbed lipid metabolism at the cellular level.

This data contributes to a better understanding of the ways MDCs affect adipogenesis and adipose tissue depots, thereby aiding the development of adverse outcome pathways (AOPs) and regulatory risk assessment.

### **1.12.P-We087 Multiomic Analysis of Tributyltin-Exposed Adipocytes Reveals Omic-Related Signatures Associated with Metabolic Syndrome**

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Metabolic syndrome is a cluster of frequently co-occurring conditions linked to various health outcomes, increasingly attributed to exposure to endocrine-disrupting chemicals (EDCs). Specifically, EDCs may impact the normal development and function of adipose tissue, once considered an inert storage depot. However, mounting evidence suggests that it is a complex and metabolically active organ with a considerable influence on the regulation of metabolism and energy homeostasis. To explore relevant effects of EDCs, Simpson-Golabi-Behmel syndrome (SGBS) pre-adipocytes were exposed to tributyltin (TBT), a known lipogenic substance, to map the omic signature associated with metabolite and transcript dysregulation. SGBS pre-adipocytes were grown to near confluence and incubated in differentiation medium for four days, followed by cultivation in maintenance medium for six days. The differentiation medium of TBT-exposed cells was additionally supplemented with 25nM TBT during the initial four days. Cells were harvested at day 10 of differentiation and TBT-exposed and differentiated controls were compared. Samples for untargeted metabolomics were analyzed using Reversed Phase (RP) and Hydrophilic Interaction (HILIC) Liquid Chromatography in positive and negative ionization modes. Transcriptomic analysis was performed using Agilent microarrays to determine differentially expressed genes (DEGs) between treatment groups. Data preprocessing, batch correction, and statistical analyses were conducted in R using *xcms*, *IPO*, *PMCMRplus*, and *xMSannotator* packages for metabolomics, and *limma* for transcriptomics. An integrated omics analysis pipeline was employed, using univariate and multivariate approaches in *mixOmics* and *MetaboAnalystR*, aimed at enhancing the depth of understanding of multiomic mechanisms. This approach highlights both individual molecular changes and their integrated effects on cellular pathways. Across analyses, perturbations in pathways linked to metabolic dysfunction, including dyslipidemia, obesity, and inflammation, were identified consistently. The consistent identification of key features and shared perturbed pathways provides a foundation for further mechanistic investigations and potentially informs strategies for mitigating the adverse effects of these exposures. Future studies will delve deeper into the adipocyte 'ome', seeking indications of susceptibility or early warning signs specific to health outcomes such as metabolic syndrome.

### **1.12.P-We088 Concomitant Investigation of Protein Expression, Neurotransmitter Levels and Locomotor Behavior in Zebrafish Larvae upon Exposure and Depuration from Neuroactive Pharmaceuticals Fentanyl and Buspirone**

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Pharmaceutical residues can contaminate aquatic environment due to incomplete removal during wastewater treatment. Exposure to these compounds can cause various effects in non-target species, such as changes in fish behavior. This could occur either due to specific disruption of normal functioning of the nervous system (since many molecular targets of neuroactive compounds are well conserved across vertebrates), or through non-specific mechanisms, such as physiological disturbance or depletion of energy reserves.

Zebrafish (*Danio rerio*) larvae are often used to test for neurotoxicity of diverse compounds by studying their effects on locomotion. However, such chemical-induced alterations of locomotor behavior were often observed to be transient (reversible) in nature, and hence their ability to reflect specific changes in the nervous system remains unclear. In order to investigate the persistency and reversibility of chemical-induced behavioral changes, we developed a test setup where behavior is first assessed at 6 days post fertilization (dpf) in zebrafish larvae continuously exposed to test chemicals from birth. To assess recovery potential, some fish are then transferred to clean water, followed by daily re-testing of behavior in depurated and continuously exposed individuals until 9 dpf.

Exposure to non-toxic levels of two neuroactive pharmaceuticals, fentanyl and buspirone, caused strong hypoactivity at 6 dpf for both compounds and in both light and dark conditions. Upon depuration, reduced activity in the dark rapidly recovered to non-exposed levels already after one day (at 7 dpf) for both substances. In contrast, reduced locomotion in the light persisted until 8 dpf for fentanyl, and did not recover even at 9 dpf (i.e., after three days of depuration) for buspirone.

To investigate molecular changes underlying these responses, we carried out concomitant molecular analyses that included global proteomics by nanoLC-MS/MS with data-independent acquisition mode and targeted metabolomics for a set of 16 neurotransmitters and precursors. Interestingly, both pharmaceuticals reduced the levels of catecholamines epinephrine and norepinephrine, and differentially affected several other metabolites. The analysis of proteomics data set is currently ongoing with the goal to identify potential protein markers which could be predictive of recovery potential in the dark and/or of persistent hypoactivity in the light, observed in response to these compounds.

### **1.12.P-We089 Proteomic Analysis of Short-Chain Perfluorinated Alkyl Substance (PFAS) Exposure in Fathead Minnows (*Pimephales Promelas*)**

*Cristina Henriques, Theresa Warriner and Denina B.D. Simmons, Ontario Tech University, Canada*

Perfluoroalkyl substances are widely used anthropogenic compounds with many structures and applications. Over the years, there has been a great concern regarding their usage as exposure can result in liver toxicity, endocrine disruption and bioaccumulation, thus resulting. As of recently, the presence of short-chain PFAS within commercial products and within the environment has risen. Current research focuses on legacy long-chained PFAS, with very little information available for the health and environmental risks of short-chain PFAS. Therefore, more information on short-chained PFAS toxicity is required as their prevalence increases. To determine the toxicity of short-chain PFAS in comparison to its' long-chained counterparts, fathead minnow (*Pimephales promelas*) were exposed to PFOS (5ug/L), PFHxS (5ug/L), PFBS(5ug/L) as well as an environmentally relevant mixture (PFOS: 110ng/L, FHxS:10ng/L, PFBS: 20ng/L) for 28 days. Post exposure, mucus, blood and brain were collected and stored at -80°C for future proteomic analysis. Muscle was also collected to determine bioaccumulation. Proteins from samples were digested with heat and formic acid, and analyzed with a LC-QTOF (Agilent 1260 LC and 6545 QTOF) using data-dependent acquisition (DDA) to create a spectral peptide library, and then MS1 filtering was performed using Skyline software and their DDA workflow. In this presentation, preliminary results from the mucus, blood and brain proteomes will be presented.

### **1.12.P-We090 Leveraging Multi-Omics Analyses to Explore the Toxicity of Urban Road Runoff Contaminants in Juvenile Salmonid Species**

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Urban roadway runoff is correlated to escalating pre-spawn mortality (PSM) events in Pacific Northwest salmon populations. Recently, a chemical derived from tires, N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-q), was isolated from roadway runoff and observed to induce acute mortality in salmonids, however sensitivity among salmonid species is varied. It is predicted that exposure to 6PPD-q will enhance salmonid sensitivity to other cooccurring contaminants commonly detected in surface waters, such as the polycyclic aromatic hydrocarbon 9,10-Anthraquinone (AQ). To determine sublethal concentrations, juvenile salmonid species (chinook, coho, and rainbow trout) were exposed to a range of concentrations of 0 – 10 µg/L 6PPD-q for 24-hours. A targeted multiple reaction monitoring (MRM) method was developed in a triple quadrupole mass spectrometer, coupled to an ultra-high-performance liquid chromatography system to quantify 6PPD-q and AQ in water samples. To better understand the mechanisms of toxicity of 6PPD-q, fish were exposed to established sublethal concentrations of 6PPD-q and AQ separately and in combination over a five-day period. AQ and 6PPD-q quantification in water tanks were validated and measured within a 15% margin of error. Coho and rainbow trout exhibited PSM symptoms leading to mortality at 6PPD-q concentrations after 24-hour exposure to 0.01 µg/L and 10 µg/L, respectively. PSM symptoms and mortalities were observed in coho and rainbow trout, but not chinook. Non-targeted mass spectrometry-based metabolomics analyses were performed on brain and liver samples from exposed and non-exposed fish. Using in-house and open-source spectral libraries we annotated 290 and 260 metabolites in the brain and liver tissues, respectively, with high level of confidence. A larger number of significant changes were observed in the liver tissues within each specie when compared to the brain tissues (Wilcoxon rank sum test,  $p < 0.05$ ). Fatty acid biosynthesis was the pathway most significantly affected by 6PPD-q exposure in coho salmon, as revealed by metabolic pathway enrichment analysis. QuantSeq 3' mRNA-sequencing transcriptomics analysis will be integrated with the metabolomics data. This study aims to clarify the impact of prioritized urban road runoff contaminants on salmonids health, contributing to a deeper understanding of the observed variations in toxicity among different salmonid species and mechanisms of action.

### **1.12.P-We091 The Use of Concentration Ranges Inducing Defense and Damage Responses as a Promising Approach to Overcome Database Gaps in Metabolite Annotation in Ecotoxicology**

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The use of untargeted metabolomic data by liquid chromatography-high resolution mass spectrometry (LC-HRMS) enables the characterization of families and functions of molecules within biological systems without making an *a priori* decision on the

molecules to be identified. However, this approach has some limitations. Indeed, the proportion of annotation of untargeted metabolomic features with the use of databases proved to be very low (~5% of the chemical signal). The trends in the dose-response model of the untargeted metabolomic feature are a biological parameter that can reflect the metabolome responses of a biological system exposed to a contaminant without the need for an annotation step. Following a meta-analysis of response trends (*i.e.* biphasic: bell- and U-shaped, monotonic: decreasing and increasing) of known biomarkers of defense and damage that we performed on 156 selected articles, 2,595 dose-response curves were interpreted from exposure experiments to inorganic and organic contaminants on 18 phyla, representing four kingdoms. The results of multinomial logistic models showed that defense biomarkers mainly describe biphasic trends, while damage biomarkers follow monotonic trends. Based on these observations, we have characterized the metabolomic response of river biofilms exposed to cobalt, using dose-response modeling, benchmark-dose calculations and characterization of response trends. The trends in the dose-response curves obtained for all untargeted metabolomic features revealed a concentration range in which the biofilms were likely to exhibit a defense response (predominance of untargeted metabolomic features with a biphasic trend) and a range in which the biofilms would instead exhibit a damage response (predominance of untargeted metabolomic features with a monotonic trend). Following these observations, we proposed a new concept of concentration range inducing defense responses (CRIDeR) and concentration range inducing damage responses (CRIDaR). This holistic approach makes it possible to use the entire chemical signal of the metabolome without being restricted by the metabolite annotation step. It can also be used to monitor the effects of exposure time with the characterization of a time range inducing defense responses (TRIDeR) and a time range inducing damage responses (TRIDaR). This approach represents a promising avenue for the development of a robust and sensitive tool for environmental risk assessment.

### **1.12.P-We092 Analyzing the Metabolic Effects of Nitrobenzene Exposure in Japanese Medaka (*Oryzias latipes*) with Consideration of Reproductive Cycle**

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The analysis of fish metabolites is a widely adopted research methodology aimed at scrutinizing diseases and detecting anomalies in diverse conditions. In this study, we seek to investigate the metabolic perspective of chemical exposure and toxicity in fish through the utilization of metabolic analysis. To accomplish this, we made a decision to investigate metabolic alterations in a manner that accounts for the potential impact of gender and reproductive cycle on metabolic changes. We chose the Japanese medaka (*Oryzias latipes*) as the experimental species due to its brief reproductive cycle that permits daily egg-laying under favorable circumstances. Furthermore, we selected a sampling method to acquire samples from both the control and experimental groups as a singular entity, involving the concentration and measurement of extracorporeal metabolites released into the media over a designated time interval. We sampled the medaka three times a day in accordance with their reproductive cycle to verify metabolic changes that are dependent on the reproductive cycle, which is characterized by daily ovulation. Nitrobenzene, a chemical commonly used in industry, was the initial chemical used in the experiment due to its potential for exposure to water systems in case of an accident and prior research highlighting its toxic effects on fish. In this study, an investigation of the impact of both the reproductive cycle and nitrobenzene exposure on the extracorporeal metabolites of medaka was undertaken. Utilizing PLS-DA for data analysis, it was discerned that the metabolites emitted by medaka underwent alterations within reproductive cycle. Moreover, a distinctive identification of metabolites that exhibited a notable decrease post nitrobenzene exposure, as compared to their pre-exposure state, indicated an encompassing influence on the reproductive cycle. To delve further into these effects, enumeration and annotation of metabolites displaying significant changes in each analysis will be conducted. Subsequently, from the metabolites influenced by sampling time and nitrobenzene exposure, we will identify biomarkers to differentiate changes attributed to the reproductive cycle and those induced by nitrobenzene exposure. By elucidating the biological characteristics of these metabolites, we aim to foresee potential transformations within the medaka.

### **1.12.P-We093 Optimization of simultaneous metabolomics and lipidomics analysis to improve reliability of multi-omics and reduce zebrafish animal use**

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Recent toxicological studies have employed multi-omics analysis to identify the underlying molecular mechanism. However, preparing individual animal samples for each omics analysis has the problem of increasing exploratory animal use. To overcome this limitation, we aimed to develop a novel approach for simultaneous analysis of both metabolomics and lipidomics. We collected 5 days post-fertilization zebrafish larvae, with pooling size of 15, 30, 50, and 100, and biological replicate of 4. Metabolites and lipids of zebrafish larvae were extracted with methyl *tert*-butyl ether (MTBE). To compare solvent effects, the metabolites of 15 larval group was further extracted with chloroform and methanol. Moreover, untargeted metabolomics employed to cover diverse metabolites, while targeted lipidomics applied to enhance identification of lipid species. Metabolomic profiles with MTBE extraction were similar to those with chloroform or methanol extraction, indicating that MTBE extraction performance was sufficient for metabolome analysis. The metabolomic profiles of 15, 30, 50, and 100 larvae contained 202, 234, 248, and 305 metabolites and 47, 45, 47, and 50 pathways. Of 54 pathways, 39 pathways were overlapped regardless of pooling size while 15 larval groups covered 86% of the pathways enriched in 100 larval group. Similarly, the lipidomic profiles of 15, 30, 50, and 100 larvae contained 274, 283, 285, and 290 lipids. The lipids identified in 100 larvae included those identified in all pooled groups, and 15 larval group was covered 94% of the lipids measured in 100 larval group. MTBE-based simultaneous extraction strategy was newly proposed for zebrafish toxicology. We showed that

both metabolomics and lipidomics can be performed at least 15 larvae and demonstrated that analyzing biomolecules of multiple layers is possible in a single sample, which will improve the reliability of omics, more importantly, reduce animal use.

### **1.12.P-We094 Lipidomic Profiling of Coral Exposed to Phthalate Demonstrates a Microplastic Pollution-Deriving Environmental Risk in the Ocean**

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Microplastics increase the bioaccessibility of chemical additives, such as plasticizers, for suspension feeders, leading to concerns regarding what detrimental effects would be induced on coral health. As a foundational role in various cellular processes, the lipidome can represent the change in the health of an organism. Untargeted lipidomic profiling of coral exposed to di(2-ethylhexyl) phthalate (DEHP) less than 2.0 µg/L for 10 days was therefore performed to gain insight into the health impacts in this study. Decreasing saturated and polyunsaturated (22:6-possessing species especially) triradylglycerols featured predominantly in coral exposed to DEHP, but increasing some of the triradylglycerols including shorter saturated and 20:5 fatty acid chains was additionally observed during the early exposure. These results indicated that DEHP can induce activation of mitochondrial and peroxisomal β-oxidation and the host-mediated storage lipid biosynthesis of symbiotic algae in the coral. Potentially expending triradylglycerols on actions such as increasing 20:5- and 22:6-possessing ether glycerophospholipids for downregulating inflammation was additionally observed in the coral host cells. Predominantly increasing membrane lipids, including betaine [such as DGCC(22:6/22:6), DGCC(16:0/28:7), DGCC(20:5/16:0) and DGCC(14:0/22:6)] and galacto- [such as MGDG(18:4/20:5) and DGDG(18:4/20:5)] lipids, that expended storage lipids as well was also observed in the coral symbiotic algae, relating with the increased light sensitivity. DEHP exposure not only induced effects similar to that regulated by peroxisome proliferators activated receptors on coral host cells but also affected the physiological conditions of symbiotic algae and potentially the interaction of symbiosis. Although coral seems unstressed directly by DEHP, some weaknesses, such as hyp immunity, would result in chronically risk coral health as highlighted by this study.

### **1.12.P-We095 Multi-Omics in Nanoplastic Research: A Spotlight on Aquatic Life**

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Amidst increasing concerns about plastic pollution's impacts on ecology and health, nanoplastics are gaining global recognition as emerging environmental hazards. This potential review aimed to examine the complex molecular consequences and underlying key toxicity mechanisms that were reported from the exposure of diverse aquatic organisms to nanoplastics. Through the comprehensive examination of transcriptomics, proteomics, and metabolomics studies, we explored the intricate toxicodynamics of nanoplastics in aquatic species. The findings raised important questions about the consistency of findings across different omics approaches, the value of combining these omics tools to better understand and predict ecotoxicity, and the potential differences in molecular responses between species. By amalgamating insights from 37 Omics studies (transcriptome 22, proteome 6, and metabolome 9) published from 2013 to 2023, the work uncovered both common and distinct toxic effects and mechanisms in which nanoplastics can affect aquatic life, also a special emphasis on zebrafish response and underlined toxicity mechanisms to nanoplastic is highlighted. Finally, recommendations were provided for advancing omics-based research on nanoplastic pollution. This comprehensive review illuminates the nuanced connections between nanoplastic exposure and aquatic ecosystems, offering crucial insights into the complex mechanisms that may drive toxicity in aquatic environments.

### **1.12.P-We096 In vivo high throughput screening for mechanism-based toxicity assessment of chemical mixtures from consumer products using *Caenorhabditis elegans* Transcription Factor RNAi**

**Elizabeth Dufourcq Sekatcheff, PhD, Siyeol Ahn, Chaein Chong and Jinhee Choi, School of Environmental Engineering, University of Seoul, Korea, Republic of (South)**

Identifying toxic effects of consumer products is challenging due to undisclosed composition of the products and the assessment of combined effects of ingredients. Indeed, toxic effects of chemical mixtures are often more complex than the simple addition of effects of ingredients. Numerous mathematical models are available to assess synergetic or antagonistic effects of chemical mixtures, but they lack empirical evidence to strongly support risk assessment and decision making. Here, we have developed an in vivo high throughput screening method to assess the toxicity mechanisms of chemical mixtures from consumer products using *Caenorhabditis elegans* model species. A set of 384 transcription factors which covers 41% of all predicted transcription factors in *C. elegans* were tested using RNAi method. The RNAi library covers a wide range of biological pathways that can be triggered upon exposure to various types of chemicals. RNAi-fed worms are then tested for various toxicological endpoints including reproduction, locomotion, or development, using automated assays. Then, the biological pathways triggered by the transcription factors significantly leading to either rescued or exacerbated toxicity are analyzed using KEGG and REACTOME databases. Downstream genes involved in these pathways are then functionally validated by measuring their expression in a dose dependent manner. This method was successfully implemented for complex

chemical mixtures such as crude oil in which worms were tested for reproductive toxicity. Several transcription factors involved in NER (Nucleotide Excision Repair) pathway led to exacerbated toxicity. Downstream genes were validated in zebrafish and showed the same dose response relationship, confirming the possibility to extrapolate to vertebrates. This approach can be used to infer molecular key events to build Adverse Outcome Pathways (AOPs) and enhance the prediction of toxicological adverse outcomes. In conclusion, this method has great potential as a fast and efficient screening method for mechanism-based toxicity assessment of chemical mixtures in consumer products.

#### **1.12.P-We097 Molecular Response of *Chironomus riparius* to Antibiotics**

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Antibiotics are widely used in human and veterinary medicine and can enter the environment by several ways. Although these pharmaceuticals have been released into the environment for many years, they are usually detected at concentrations below than those known to cause acute toxicity to organisms. Indeed, antibiotics have received particular attention, mainly because of the ability of bacteria to develop resistance against them. While these biologically active compounds are designed to interact with microorganisms, the continuous introduction of antibiotics into the environment likely has an impact on non-target organisms living in aquatic systems.

Standard toxicological tests may fail to detect the adverse effects of antibiotics, however, new methods can give an insight into the pathways and physiological responses affected by them. *Chironomus riparius* is a dipteran with aquatic larvae widely used in toxicology tests. This species is abundant in the freshwater ecosystems, acts as an important component of many food chains, moreover it is sensitive to different toxicants. Currently, little is known on the effects of antibiotics on this aquatic insect at the molecular level.

In this study, the gene expression profile of *C. riparius* in response to different groups of antibiotics (aminoglycosides, fluoroquinolones and penicillins) was assessed. Fourth instar larvae were exposed to 0.001, 0.1 and 10 mg/L of each antibiotic for 24 and 72 h. The expression of genes involved in hormonal regulation, detoxification mechanisms, stress response and DNA repairing were analyzed. Results showed that all antibiotics altered the mRNA levels. Three of the four antibiotics (amoxicillin, neomycin and levofloxacin) caused downregulation at 24 h and an increased transcription of genes at 72 h. While genes affected by gentamycin showed an opposite trend.

These findings indicate that the transcriptional activity of endocrine related, cellular stress, detoxification and DNA repair genes was altered by antibiotics in *C. riparius*. Gene expression analysis has been shown as a useful tool to provide information on the underlying mechanisms of toxicity for these pharmaceuticals. Additional research is needed to better understand the impacts of antibiotics on this species.

#### **1.12.P-We098 "Understanding impact of BEMT on larval zebrafish: Thyroid hormone disruption, transcriptome profiling and implications for early life development"**

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Due to the health risks associated with ultraviolet radiation, the use of sunscreen is commonly recommended. Bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT) serves as an organic UV filter and UV stabilizer in sunscreens. Its extensive use and frequent detection in sediment and freshwater have raised concerns regarding potential environmental health implications. However, limited information exists regarding its toxicological characteristics, notably its impact on endocrine disruption.

In this study, we conducted a 5-day exposure of embryo-larval zebrafish (*Danio rerio*) to a range of BEMT concentrations (0.01, 0.1, 1µM). Following the exposure, we evaluated its effects on thyroid hormone disruption and developmental toxicity. Additionally, we performed RNA-seq analysis to identify differentially expressed genes (DEGs) and to elucidate affected genes and pathways.

Our findings revealed a dose-dependent decrease in thyroid hormone levels in larval fish along with down-regulation of genes associated with the hypothalamic-pituitary-thyroid (HPT) axis. Moreover, exposure to higher BEMT concentrations resulted in a notable decrease in swim bladder size in larval zebrafish, indicating potential thyroid hormone disruption. RNA-seq analysis unveiled a significant number of DEGs, notably the downregulation of *ugt1ab* and *hhex* genes. Confirmatory qPCR analysis suggested reduced thyroid hormone metabolism, aligning with the observed decrease in thyroid hormone levels in the fish.

Furthermore, KEGG and GO analyses indicated that upregulated DEGs were primarily involved in neurological pathways, while downregulated DEGs were associated with lipid metabolic pathways. These observations suggest potential disruption in nervous system development and lipid metabolism due to BEMT exposure, in addition to thyroid hormone disruption. However, the correlation between alterations in neurodevelopment and lipid metabolism with thyroid disruption requires further investigation. Given the limited understanding of BEMT's toxicity despite its widespread use, our study underscores the necessity for comprehensive investigations into its endocrine and various other effects, particularly during early stages of life.

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### 1.13 The Endocrine Disrupting Properties of Challenging Substances: How to Solve Testing and Interpretation Issues?

#### 1.13.T-01 How to screen UVCBs endocrine activities using OECD guidelines: a case study with essential oils

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UVCBs are composed of numerous molecules with different physical and chemical properties influencing their solubility in water, some being easily soluble while some others are practically insoluble.

Assays performed on aquatic eleuthero-embryos of Japanese medaka fish (*Oryzias latipes*) or amphibians (*Xenopus laevis*), require test items to be fully solubilized in aqueous water-based test media. These assays have been developed to identify endocrine active chemicals or mixtures e.g., the XETA assay (OECD TG 248), the RADAR assay (OECD TG 251) and the REACTIV assay (OECD draft TG under review).

We will aim to describe the critical steps of the methodologies to successfully assess UVCBs using *in vivo* models at an *in vitro* scale.

Prior to definitive tests, solubility in test media needs to be determined for each UVCB. If the test item is not fully soluble in the test medium alone, further solubility assays are performed according to the OECD Guidance Document 23 using organic solvent to facilitate solubility. A number of organic solvents including DMSO and ethanol have been shown to be inert on the estrogen, androgen and thyroid axes, as well as steroidogenesis when using the assays listed above.

Subsequently, a critical step is determining of the maximum tolerated concentration (MTC). The MTC is determined by carrying out a range-finding trial performed in the conditions of the definitive test. The highest concentration tested in the range-finding experiment is set by the solubility limit or 100 mg/L, whichever is the lowest, and generally four test concentrations are added following a separation factor  $\leq 10$ .

It is also important prior to testing UVCBs to evaluate the ability of the assay to correctly identify the net effects of several active chemicals in a complex environment, i.e., in the presence of multiple chemicals that are inert in the assay.

Finally, using an endocrine system to assess an endocrine activity is critical. Eleuthero-embryos are sensitive to multiple modes of action, taking into account chemicals acting through nuclear receptors, transporters, hormonal metabolism etc. The specificity of the endocrine response is ensured by reading out expression of a reporter gene under the control of a gene promoter which is directly and specifically regulated by the endocrine axis in question.

Examples of the testing of essential oils and their constituents will be presented to illustrate the importance of these methodological steps.

#### 1.13.T-02 Assessing the endocrine disruptor potential of hydrocarbon UVCBs

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New hazard classes are being introduced under the EU Classification and Labelling (CLP) regulation to cover endocrine disruption (ED). In addition, data requirements for assessing ED are planned as part of updates to the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals regulation (REACH). ED assessments are complex and data intensive, and multiple mechanisms of action, including effects occurring in the presence of general systemic toxicity, can affect the outcome. Draft CLP guidance for ED does not currently cover UVCBs (substances of unknown or variable composition, complex reaction products or of biological materials), but it is expected that both whole substance data and data for representative constituents will be required to conclude on ED classification.

Here we describe an initial ED data gathering exercise for example UVCBs in a petroleum stream category. Some whole substance *in vivo* data were available for mammals and screening level data based on quantitative structure–activity relationship (QSAR) and *in vitro* ToxCast bioassays was gathered for 25 selected representative constituents covering eight hydrocarbon classes. Many of the constituents were found to be outside the QSAR applicability domains of the methods. Also, despite there being *in vitro* data available for 17 constituents, only data for three were acceptable because analytical data could not adequately confirm the presence of the test item for 14 of the substances, most of which are volatile.

The acceptable *in vitro* data were useful for prioritising constituents for further investigation, with e.g. biphenyl assigned as low priority, and phenanthrene and fluoranthene both assigned high priority. The other screening data were however of limited value, with no scope for ruling out ED activity or considering whether ED activity might differ within or between hydrocarbon classes. Further work is needed to develop more reliable QSAR models and *in vitro* data for hydrocarbons. However, given the difficulties with testing already observed in the available dataset, it is currently unknown whether the battery of proposed *in vitro* tests for ED that are expected to be introduced under REACH will be suitable for testing certain hydrocarbon constituents (e.g., volatiles) or whole petroleum substances.

### 1.13.T-03 How to Effectively Assess the Endocrine Disruption Potential of Metals Under the EU CLP Regulation?

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New hazard classes for endocrine disruption (ED) have been introduced to the EU's Classification Labelling and Packaging Regulation (CLP). Substances may be classified as 'known or presumed to be endocrine disruptors' (Category 1) or 'suspected to be endocrine disruptors' (Category 2) in humans or in the environment, with the differentiation between the categories based on the strength of available evidence. This presents a new challenge for the hazard classification of chemicals that are naturally involved in, or interact with, the endocrine system, particularly where supporting data from regulatory studies is lacking or inadequate.

Metals and inorganic metal compounds have specific properties that complicate their assessment. This is especially the case for essential elements which have a function in a wide variety of endocrine and non-endocrine physiological processes. New guidance is being developed by ECHA on how to assess substances for ED under CLP. A recently published framework describes the three main types of interactions metals can have with the endocrine system: indirect effects, endocrine modulation, and endocrine disruption. This project developed a series of worked examples to demonstrate how this framework can be integrated into and improve the guidance on how to evaluate metals to allow appropriate hazard classification in accordance with CLP Regulation.

Three examples were developed based on realistic and theoretical datasets. These data varied in reliability, included non-standard endpoints and/or non-standard test species. The assessment considers three criteria for endocrine disruption: adverse effects, endocrine activity, and a biological plausible link between the two, and uses a weight of evidence (WoE) approach to conclude the appropriate hazard classification. By giving weight in the evaluation to data depending upon their reliability, the WoE approach helps in identifying the most plausible toxicity pathway that is occurring, and whether this is driven by ED or by an alternative mechanism (e.g., endocrine modulation or indirect effects on the endocrine system). This integration of the metals framework provides a better understanding of the complexity of metal interactions in endocrine systems. This framework, and the examples presented here, can complement ECHA's guidance on CLP, particularly with the WoE approach, to support accurate hazard classification of metals for ED.

### 1.13.T-04 The Challenges of Distinguishing Non-Endocrine from Suspected Endocrine Responses: The Precautionary Tale of Copper

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Experimental design plays a vital role in ecotoxicological endocrine disruption (ED) studies as it helps to distinguish between direct endocrine-related effects and other mechanisms that may involve indirect or compensatory endocrine responses. In both fish and amphibians, non-endocrine factors such as bioenergetics, dietary iodine availability, systemic toxicosis, oxidative stress, and treatment-induced stress must be considered to avoid any misinterpretation. A study was conducted to evaluate whether copper exposure during pre-metamorphic development in *Xenopus laevis* resulted in systemic toxicity that affects early development, growth, and metamorphic processes, independent of the HPT axis. Starting at developmental stage 10, frogs were exposed to copper at test concentrations of 3.0, 9.0, 27.2, 82.5 and 250 µg Cu/L. The primary endpoints included mortality, developmental stage, malformation, hindlimb length, growth (snout-vent length and wet body weight), and histopathology. The study revealed that whilst there was marked developmental delay in frogs exposed at 27.2 and 82.5 µg Cu/L concentrations, this was the result of systemic toxicity that occurred early in development, prior HPT-driven metamorphosis. This delay was not indicative of ED. In order to assess the impact of feeding on the consistency of Cu levels at low treatment concentrations, a 24-hour test was conducted using a simulation of a flow-through diluter system. Results indicated that normal feeding increased total Cu levels by 1 to 8 µg/L total copper reducing the ability to control copper exposures at low concentrations. Finally, a study is currently being conducted to evaluate the potential impact of copper exposure on reproductive performance in adult zebrafish (*Danio rerio*). This study will assess the effect of oxidative stress on the reproductive fecundity over 21-days. Sub-sampling at different timepoints will be done to measure biomarkers of oxidative stress in the gonad and liver. Reproductive endpoints will include fecundity measurements (eggs produced/female/day and fertilization daily), gonado-somatic index, liver-somatic index, plasma vitellogenin, and gonad, liver, and liver histopathology at day 21. This fish reproduction study will assist in understanding the link between non-ED toxicity, such as oxidative stress, and disturbance of the HPG axis by measuring both simultaneously.

### 1.13.T-05 Population Relevance of Endocrine-Mediated Effects of Pesticide and Biocide Substances – A Way Forward

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Population modelling, field studies and monitoring approaches have all been proposed for assessing the effects of Endocrine Disrupting Chemicals (EDCs) at the population level for non-target vertebrates, but how these approaches should be used in the regulatory EDC hazard assessment in Europe is unclear and not detailed in the relevant Guidance Document by ECHA &

EFSA (2018). Science-based recommendations from this ECETOC expert group have been developed and presented here with the objective of addressing this shortcoming. Starting from a literature review focused on identifying published approaches assessing the population relevance of effects from EDCs, four guiding principles were developed for assessing the effects of EDCs at the population level, all together forming the 'PIER' framework: 1) Phrase (the regulatory question) - the definition of protection goals and choice of focal species (and models); 2) Implement - the individual level endpoints to be considered, the magnitude of effect to be imposed, for what duration effects should be imposed and whether individuals repairing the damage from exposure should be included; 3) Evaluate - the population-level endpoints to be considered, what threshold to set for defining an adverse effect and whether population recovery should be considered; 4) Report - the requirements for documenting the whole process. Examples for each relevant modelling- and field- case study category from the literature that are generally consistent with the PIER framework were also developed. For the selected case studies, we suggest alterations in the modelling approach and study design which would result in studies that would fulfil the PIER framework requirements and be ready for use in a regulatory context. The PIER framework presented here aims to provide a consistent approach to the population relevance assessment of EDCs based on the criteria set out in Regulations (EU) No 2017/2100 and 2018/605 and may be used to design and evaluate an appropriate modelling, monitoring or field study for any chemical to determine the population relevance of any adverse effects observed in the laboratory. None of the studies identified by the literature review comply entirely with the PIER framework we have developed, thus we suggested alterations in modelling approach and study design which would result in studies that would fulfil the PIER framework. It would be desirable to see examples that fully comply with the approach in the future.

### **1.13.P The Endocrine Disrupting Properties of Challenging Substances: How to Solve Testing and Interpretation Issues?**

#### **1.13.P-Tu035 Endocrine Disruptors – Hazard Classification Under CLP And New Challenges With Aquatic Tests**

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New EU regulation set classification of Endocrine disruptors (ED) for human health and ED for the environment. These classifications require thoughtful weight of evidence assessment of all relevant data. The draft CLP guidance according to which the chemicals should be assessed is available, and its final version is to be released in the Q2 2024. The draft guidance contains some approaches which were recently discussed in the scientific community and will be globally tested for the first time in the regulatory practice. The new ED REACH requirements and restriction mechanism is expected to be legalised by new Commission after EU election. This is unbalancing an expected regulatory timeframe as the required data sets for the ED evaluation should be defined in REACH whereas the way how to assess without data requirements is formulated in the CLP draft guidance. Due to variability of the REACH chemicals some ED methodologies will be difficult to be tested. Regarding aquatic toxicology, the properties of challenging chemicals and how to address them are described in the OECD 23. This guidance is however not specifically designed for the more robust aquatic ED tests as FSTRA, AMA, XETA, EASZY assay. Regarding assays as XETA and EASZY, that work with genetically modified organisms, additional properties of chemicals interfere with the receiving the correct results which are not defined in OECD 23. First, it is autofluorescence of tested chemicals. Positive endocrine activity in these tests is recognised by decrease or increase of fluorescence. Autofluorescent chemicals bring seemingly dose dependent response indicating ED activity of a chemical. Further, UV activated toxicity of tested chemical can bring almost instant mortality of tested organism. Next to these physicochemical properties, the embryonal model can be sensitive to different toxic mode of action in complex mixture of chemicals than seen in previous testing. These challenges bring more demands into the planning and designing the tests and whole ED testing strategy. Especially since the ED identification of chemical leads to ban of chemical substance in biocidal, plant protection and probably in future REACH Regulation. Any falls positive results or impossibility to perform a planned study within short regulatory timeline is extremely difficult. It is therefore desirable, that difficult substances should be well anticipated and well described in the future REACH requirements or updated CLP guidance.

#### **1.13.P-Tu036 Endocrine Disrupting Assessment for industrial chemicals, biocides and plant protection products in the EU – Summary, challenges and improving points**

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The endocrine system is a complex network, making endocrine disrupting assessment challenging. The disparities among relevant (new) regulations for different types of substances/products further complicate the process, especially for people without a scientific background. Moreover, for certain substances (e.g. UVCB), the required testing(s) for the endocrine disruptor endpoint can pose a significant challenge, even for experts in this field. While bridging the science and regulation to improve the endocrine disrupting assessment and testing strategy, especially for “difficult” substances, it is necessary to have an overview of the current frameworks, the challenges in each step of the process and the potential solutions.

Our work provides a comprehensive overview of the current EU regulations concerning endocrine-disrupting properties in industrial chemicals, biocides, and plant protection products. Endocrine disrupting assessment has been a mandatory requirement for the approval of biocides and plant protection products in the EU since 2018. These assessments encompass both active substances and non-active substances, such as co-formulants, safeners, and synergists, though distinct regulations apply to each. In April 2023, the delegated regulation on the Classification, Labelling, and Packaging (CLP) of chemicals came



into effect, introducing a new hazard class for endocrine disruptors. Since then, endocrine disrupting assessment has gained significance in the regulation of industrial chemicals under the 'REACH' framework.

This overview not only provides a visual summary of the criteria for evaluating endocrine disrupting endpoints, but also pinpoints areas where enhancements in the assessment process are feasible. For instance, following the available guidance on screening the endocrine-disrupting characteristics of co-formulants can lead to a substantial workload and divergent interpretations among various stakeholders involved in the evaluation process. Furthermore, the environmental endocrine-disrupting properties were not sufficiently covered in the evaluation, which is expected to improve when new (eco)toxicity testing guidelines for ED identification lead to more available data. Our study also delves into the methods and challenges linked to conducting endocrine disrupting assessment for UVCB as a co-formulant in biocidal products and industrial chemical (under REACH regulations), offering a regulatory perspective as a case study.

### **1.13.P-Tu037 Challenges and Recommendations in Assessing Potential Endocrine Disrupting Properties of Metals in Aquatic Organisms**

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New tools and refined frameworks for identifying and regulating endocrine disrupting chemicals (EDCs) are being developed as our scientific understanding of how they work advances. Although focus has largely been on organic chemicals, the potential for some metals to act as EDCs in aquatic systems is receiving increasing attention. Metal interactions with the endocrine system are complicated as some metals are essential to physiological systems, including the endocrine system, and non-essential metals can have similar physio-chemical attributes that allow substitution into or interference with these systems. Consequently, elevated metal exposure could potentially cause endocrine disruption, but can also cause indirect effects on the endocrine system via multiple pathways or elicit physiologically appropriate compensatory endocrine mediated responses (endocrine modulation). These latter two effects can be confused with, but are clearly not, endocrine disruption. In this paper, we provide several case studies that exemplify the challenges encountered in evaluating the endocrine disrupting (ED) potential of metals followed by recommendations on how to meet them. Given metal toxicity can be caused by multiple modes of action (MoAs), we recommend assessments use metal-specific adverse outcome pathway networks to ensure accurate causal links are made between MoAs and effects on the endocrine system. We recommend more focus on establishing molecular initiating events for chronic metal toxicity as these are poorly understood and would reduce uncertainty regarding the potential for metals to be EDCs. Finally, more generalized MoAs such as oxidative stress could be involved in metal interactions with the endocrine system, and we suggest it may be experimentally efficient to evaluate these MoAs when ED is inferred. These experiments, however, must provide explicit linkage to the ED endpoints of interest.

### **1.13.P-Tu038 Experiences in developing the Amphibian Metamorphosis Assay for regulatory testing – a CRO perspective.**

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With the new EUH-statements and new hazard classes for Endocrine Disruptors (ED) included in the latest version of the CLP Regulation, there is an increasing need for *in vivo* tests capable of identifying ED properties.

To support global chemical risk assessments for our clients, we have established and maintained a *Xenopus laevis* colony and investigated the feasibility of amphibian testing at our laboratory at Scymaris Ltd. Frogs are housed in groups of 6 individuals and spawned every three months on rotation to ensure good egg production and the capacity to start studies monthly.

The Amphibian Metamorphosis Assay (AMA; OECD 231) is a Conceptual Framework level 3 screening assay to identify substances interfering with the hypothalamic-pituitary-thyroid (HPT) axis. Amphibian metamorphosis is modulated via thyroid dependent processes and *X. laevis* is a well-studied and easy to keep under laboratory conditions amphibian model. The observational endpoints for this assay are hind limb length, snout to vent length (SVL), developmental stage, wet weight, thyroid histology and daily observations of mortality.

Sodium perchlorate, which impairs thyroid function by interacting with the sodium iodide symporter (NIS) responsible for iodide uptake in the thyroid gland and thyroxine, the endogenous hormone synthesized by the thyroid gland were used as the two reference substances.

Temperature and feed were adjusted during the pre-exposure phase to ensure tadpoles reached the required developmental stage (NF 51) at test start. Feeding during the exposure phase was decreased to keep the percentage of tadpoles showing developmental stage beyond NF 60 at test end below 20%. Significant treatment effects for developmental stage were determined from the step-down Jonckheere-Terpstra test as well as the multi-quantal Jonckheere test and results compared. Additional test replicates were continued to cover endpoints in the extended AMA test. Different blood sampling methods

were also tested and plasma volumes gained were used to investigate the feasibility of measuring plasma T3 and T4 at this timepoint.

Both flow-through studies passed the guideline validity criteria and all performance criteria related to mortality rates and the control group developmental stage (median and distribution) were fulfilled.

### **1.13.P-Tu039 Lessons learned running a Zebrafish Extended One Generation Reproduction Test as part of a validation exercise**

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An update to the Classification, Labelling and Packaging (CLP) regulation to include new hazard classes including those relating to Endocrine Disruption (ED) aligns this with the existing regulations for biocides and plant protection products whereby substances require assessment for their ED properties as part of their environmental risk assessment.

The OECD provides guidance for ED testing under a conceptual framework with tests being assigned a level based on the comprehensiveness of the testing. The highest level 5 tests include multigeneration testing, such as the Medaka Extended One Generation Reproduction Test (MEOGRT; OECD 240). This test has been reported as difficult to perform, with tests often failing to meet validity criteria, meaning interpretation of results can be difficult. An alternative EOGRT option using zebrafish (*Danio rerio*; ZEOGRT) was proposed in 2015 and is currently at the ring test stage of validation as part of an OECD workplan.

We report on the lessons learned while conducting a ZEOGRT test as part of the validation process. As for other chronic fish studies (OECD 229, 234, 240), the consistency of the test substance analytical chemistry is a proposed validity criterion ( $\pm 20\%$  of mean measured). Therefore, following initial dosing trials, Prochloraz was selected as the test reference substance over Tamoxifen citrate as it had more reliable dosing and solubility. Triethylene glycol was utilized as a carrier solvent to aid dissolution, and both a dilution water control and solvent control were employed. The test design, adapted from the MEOGRT procedure, began with a filial 0 ( $F_0$ ) parental adult group in spawning status, followed by a complete  $F_1$  generation derived from the parental eggs and finally a  $F_2$  generation derived from the  $F_1$  eggs, limited to the hatching period. Endpoints include hatch success, post-hatch survival, fecundity, fertility, length, weight, sex ratio, vitellogenin and gonad histopathology. Practical experiences running the test are shared with particular reference to the successful initiation of the  $F_1$  generation, meeting the required hatch and survival validity criteria, maintaining true replication in test vessels, the practicalities of  $F_1$  daily breeding and the timing of evaluation of  $F_1$  egg numbers prior to the initiation of the  $F_2$  generation.

The data obtained in this test will be analysed to investigate the applicability and replicability of the test design and will assist in refining the test method.

### **1.13.P-Tu040 Control Performance of Medaka Extended One Generation Test Designs**

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Evaluation of endocrine activity in humans and wildlife involves conducting specific assays to evaluate relevant pathways. Multiple *in vivo* test guidelines (TGs) have been validated for mammals, amphibians, and fish, focusing on the estrogen, androgen, thyroid, and steroidogenesis pathways. These assays require the use of a substantial number of laboratory animals, and their outcome can lead to significant regulatory actions. It is therefore critical that the assays are sufficiently reliable and robust.

One such assay, the medaka extended one generation reproduction test (MEOGRT), is designed to provide comprehensive data on adverse effects and endocrine-relevant endpoints for key aspects of the fish life cycle. Potential adverse effects on population-relevant parameters are assessed including survival, growth, development sex ratio (phenotypic vs. genetic) and reproduction. Parameters are also measured which provide mechanistic information and linkage between results from other studies where there is evidence for a chemical having the potential to interact with endocrine pathways.

The current work aims to use historical control data (HCD) to better understand the test performance and endpoint relevance and facilitate understanding of variability within the method as well as data interpretation now that these assays are being more widely conducted. Control data were collated for 25 control groups from 24 independent studies conducted following the MEOGRT TG or providing similar data as in the MEOGRT. The data were assessed across 17 biological validity criteria specified within the TG to determine how often the relevant data were reported and how often control groups meet these criteria. Reliable HCD ranges are being developed for the core study endpoints and the associated validity and performance criteria. Additionally, the HCD is being explored to investigate cross-laboratory and study differences, sources of variability, and the power of the test design.

In summary, this retrospective data analysis will form a knowledge base that could be used to determine what is realistically achievable in terms of the assay performance/validity criteria and inform improvements to the test performance and interpretation of data, which will in turn reduce the instances where these studies may need to be repeated.

This abstract does not necessarily represent US EPA policy.

### **1.13.P-Tu041 Optimising Concentration Setting for In Vivo Endocrine Screening Assays with Aquatic Vertebrates**

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There is growing regulatory demand for fish and amphibian *in vivo* data to support identification of chemical-induced endocrine activity. To ensure that endocrine-specific effects are detected and aid data interpretation, improvements to concentration setting guidance are needed. Determining the most appropriate highest concentration – or maximum tolerated concentration (MTC) – allows for clear distinction of effects attributable to endocrine-related activity from those of systemic or secondary toxicity that is not directly endocrine mediated. This is challenging because biological effects may depend on the life-stage and species tested. Currently, the MTC is inconsistently defined within the different OECD test guidelines. This work aims to provide a consensus approach to MTC setting for fish and amphibian endocrine studies by developing a strategy for concentration setting using existing data.

A database using results from regulatory fish acute and chronic toxicity studies and corresponding endocrine screening studies (fish short term reproduction and amphibian metamorphosis assays) was established for 32 pesticide active substances. The results of a meta-analysis performed to assess the effectiveness of using data from available acute and chronic studies in setting the MTC for the endocrine screening studies will be presented. This entails examining a) how MTCs derived using different approaches (1/3<sup>rd</sup> or 1/10<sup>th</sup> of the fish 96 h acute LC<sub>50</sub>) relate to effects seen in the acute studies; b) how MTCs derived from results of standard acute and chronic studies relate to the highest concentrations tested in endocrine screening studies; c) how the acute and chronic toxicity outcomes relate to the findings in the endocrine screening assays, *i.e.* whether overt toxicity was observed in the selected test concentrations and if the (remaining) concentrations were sufficient to allow for detection of endocrine activity.

The results will be considered in development of a weight of evidence approach which can be utilised to optimise the setting of the MTC for aquatic vertebrate endocrine tests. Ultimately this work will support the interpretation of data from endocrine screening studies and reduce the instances where studies may need to be repeated, or higher tier *in vivo* tests are triggered unnecessarily. Reducing instances of overt toxicity will also result in the welfare benefit of reducing severity of effects experienced by animals in such assays.

### **1.13.P-Tu042 Monitoring endocrine disrupting chemicals (EDCs) in wastewater using bioassays: a proof-of-concept from Quebec, Canada**

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Endocrine disrupting chemicals (EDCs) are compounds which can interact with the hormonal system and generate adverse effects (e.g. reproduction, growth). EDCs have been detected in municipal and industrial wastewater for many years. Despite their impacts, no countries have established maximal endocrine disrupting activity discharge criteria for receiving environments, including in Canada. The aim of this project was to use New Approach Methodologies (NAMs), especially *in vitro* EDC-based approaches, for resource water management. *In vitro* bioassays have the advantage of integrating mixture effects which allows lower limits of detection and detect unknown chemicals. The designed bioassay-based approach was constituted of two transactivation assays for the receptors of estrogen (ER) and androgen (AR), testing for estrogenic, anti-estrogenic, androgenic, and anti-androgenic activities. To validate the approach, two subsequent experiments were conducted on a municipal and an industrial effluent. *In vitro* data were compared to *in vivo* experimental data ran in parallel. Both experiments showed that fish reproduction was significantly impacted by the municipal and an industrial effluent and this could be linked to detected transactivation assay activations. The purpose of EDC screening in municipal and industrial effluents is to inform the different actors on steps to take to manage and to mitigate the quality of the effluents being released into Canadian ecosystems. For example, these *in vitro* assays could be helpful to evaluate wastewater treatment efficiency and agricultural run-off. Finally, this project provided basis to a first proof-of-concept for the use of bioassays to monitor EDCs in wastewater in the province of Quebec, Canada.

### 1.13.P-Tu043 AnthroDrugs-EDC: Personalized Endocrine Disrupting Chemical (EDC) Toxicology through Population Genetics

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Endocrine disrupting chemicals are a major concern for human and environmental toxicology because they can cause disruptions in reproductive function, foetal development, thyroid function, and increase the risk of reproductive cancers in men and women. They are also linked to infertility, early puberty, obesity, diabetes, and various cancers" (Mendes 2002, Klanova 2019)

Currently marketed and new drugs can be endocrine disrupting chemicals (EDCs), which is a concern in drug design and development (Soverini 2018).

Personalized medicine is one of the elements of the *P4* medicine of the future, and has already shown its ability to improve patients' lives in an individualized way (e.g. Soverini 2018, Gambardella 2020). However, a series of limitations hinder its widespread implementation in the immediate or short term, which has led to the development of different methods of population stratification (McGuire 2020, O'Hanlon Cohrt 2021, Litman 2019). One of these approaches is stratification based on the biological ancestry of patients, which has seen significant progress in recent years thanks to developments in DNA and ancient DNA analysis.

Stemming from our team's previous work in personalized medicine and drug development (e.g. Font-Porterías 2021, Bhat-Ambure 2023), in this work, we propose to apply "personalized toxicology" for the optimization of drugs with potential EDC by leveraging the human population genetics knowledge about genes related with endocrine disruption. We analyse drug-related genes (DRGs) which are also related to the physiological response caused by endocrine-disrupting chemicals (EDCs), thus allowing for newer, optimized, safer-by-design drugs for all human populations. This contributes to avoiding the current pitfalls of a drug development process specifically focused on people of European descent.

In particular, we analyse a dataset of EDC-DRGs (drug toxicity-related genes) collated from the literature, describing their variability in populations, and searching for potential signatures of natural adaptive selection in specific continental regions (Africa, East Asia, South Asia, Europe, and America). For selected genes, we analyse how the population variability can affect the toxicity experienced by individuals when taking the drugs involved (e.g. affected adverse outcome pathways (AOPs) and molecular initiating events (MIEs) or key events (KEs), and whether drugs can be improved in this respect.

### 1.13.P-Tu044 The Endocrine Disruption Potential of Isoeugenol and Altrenogest in *Daphnia magna*

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Industrial discharges, intensive agriculture, and improper waste disposal contribute to the presence of harmful chemicals in water bodies. Many substances, such as medicines or industrial chemicals, are created for specific purposes. Still, what happens when these substances enter the environment and affect non-target organisms is often not fully understood. Endocrine-disrupting chemicals (EDCs) are human-made or natural substances capable of affecting the endocrine system in humans and animals, leading to adverse effects, such as alterations in fertility and offspring output and disturbance of the immune system, among others.

This study aims to investigate and understand the potential endocrine-disrupting effects of two compounds, Isoeugenol and Altrenogest, in *Daphnia magna*. Isoeugenol is a natural organic compound and is renowned for its aromatic properties. Altrenogest is a synthetic progestin, and its application is in veterinary medicine. As a starting point, the *in vitro* screening assay, YES, was conducted to determine the ability of both compounds to bind to the human estrogen receptor in the *Saccharomyces cerevisiae* yeast. After this confirmation, the two Technical Guidelines from OECD 202 and 210 were used to assess the immobilization and reproduction of *D. magna* exposed to both compounds and the respective ecotoxicity endpoints (NOEC, LOEC, LC50 and EC50) were derived. Generally, altrenogest was shown to be more toxic than isoeugenol (e.g. LC<sub>50</sub> of 0.793 mg/L vs LC<sub>50</sub> of 4.39 mg/L, respectively), with reproduction impairment following a similar pattern at very low concentrations. By integrating the results from the YES assay with those from the daphnia reproductive output can highlight the effects of daphnia as an endocrine disruptor. This must be confirmed by future studies, under lower levels of biological organization (e.g. NAMs), to identify mechanisms underlying this process. The present study highlights the importance of addressing the environmental impact of these substances more comprehensively to ensure safety and sustainability in their use.

### 1.13.P-Tu045 Endocrine Activity of Bisphenol A and Eleven Structural Analogues

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Bisphenol A (BPA) is currently one of the most used synthetic chemicals in the production of a wide range of plastics. Due to its diverse endocrine disrupting potential alternative bisphenols, also referred to as analogues, were developed. Since the EU

identified BPA as a substance of very high concern it is required to be substituted with safer, more sustainable alternatives, increasing the production of the various different bisphenol analogues. While the toxicity of BPA is well studied, (eco)toxicological effects of the bisphenol analogues are largely unknown. However, the similar molecular structure of the analogues suggests comparable toxicological effects. This study evaluates different endocrine endpoints of eleven bisphenol analogues (BPB, BPE, BPF, BPS, BPZ, BPAF, BPAP, BPBP, BADGE, TBBPA, and TCBPA) compared to the reference BPA *in vitro*. The test battery includes five different yeast reporter gene assays (estrogenic, anti-estrogenic, androgenic, anti-androgenic, dioxin-like) to assess the endocrine potential or dioxin-like activity of the substances. Almost all bisphenols demonstrated notable estrogenic and anti-androgenic activity *in vitro* in a comparable range to the reference bisphenol A. Some analogues even exceeded the endocrine potential of BPA. No androgenic or dioxin-like activity was recorded in the tested concentration range for any of the twelve substances. The current work contributes to the insufficient body of literature concerning the risk assessment of bisphenol analogues.

### 1.13.P-Tu046 Urinary Concentrations of Bisphenols in Young Women from Southeastern Spain: An Exploratory Study.

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Bisphenol is a chemical that has been involved in health problems such as diabetes, cardiovascular diseases and especially in reproductive damage. This product is excreted by urine, the sample most widely used for the biomonitoring of bisphenols. The main objective of this study was to evaluate the urinary concentrations of four bisphenols (BPA, BPF, BPE and BPC) in 297 young women from Southeastern Spain, as well as potential socio-demographic and lifestyle factors associated with these bisphenols concentrations. The presence of some endocrine disruptor chemicals, such as bisphenols, has been poorly characterized in young women, particularly bisphenols others than BPA. Total bisphenols (sum of free and glucuronidated forms) present in urine were extracted using a dispersive liquid-liquid micro-extraction procedure and analyzed using an Agilent 6890N gas chromatograph coupled to an Agilent 5973 quadrupole mass selective spectrometer. The column used was an HP-5MS capillary column (30 m length x 0.25 mm internal diameter and 0.25 µm film thickness). A database was assembled with socio-demographic and dietary information and urinary bisphenol concentrations. Socio-demographic and lifestyle data was collected with a questionnaire administered face-to-face by trained field staff. Variables considered as independent variables in this study were: age, body mass index, smoking habit, regular alcohol intake, physical activity, oral contraceptives, recent self-perceived exposure to paint, glue, etc, dietary information included origin of drinking water and frequency of consumption of fish, canned food, dairy products, eggs, meat, vegetables, legumes and fruit. BPA was the bisphenol most frequently detected (95.6%), followed by BPF (45.1%), BPC (20.3%) and BPE (11.5%). Mean concentration (± standard deviation), in ng/ml, obtained were 5.10 (± 6.00) for BPA, 2.23 (± 3.83) for BPF, 0.20 (± 1.26) for BPE, and 1.11 (± 3.28) for BPC. The main hypothesis is that urinary concentrations of bisphenols are associated with several socio-demographic and lifestyle factors in young women from Southeastern Spain. The exposure to bisphenols, well-known endocrine disruptors, is a modifiable risk factor. If urinary bisphenols concentrations are higher compared with other populations and some socio-demographic and lifestyle factors area associated with those levels, potential public health actions could be put in place in order reduce those potential harmful exposures in young women.

### 1.13.P-Tu047 The endocrine disrupting potential of selective serotonin reuptake inhibitors

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The use of selective serotonin reuptake inhibitors (SSRIs) has increased steadily over the last decades and is expected to rise further. Hence, the residues of SSRIs and related antidepressants accumulate to concentrations in the µg·L<sup>-1</sup> range in surface waters. Although not perceived as classical endocrine disrupting chemicals, numerous studies have reported effects of SSRIs on steroidogenesis and steroid hormone signalling in fish. Using a reporter gene assay with the three nuclear oestrogen receptors (Esr1, Esr2a, Esr2b) and the two membrane oestrogen receptors (Gper1 and Gper1-like) from the European seabass, *Dicentrarchus labrax*, fluoxetine (FLX) displayed antiestrogenic activity on Esrs in the presence of 17β-oestradiol (E2) as well as oestrogenic activity on the two Gpers without any interaction with E2. Furthermore, fish scales exposed to FLX had a mineral turnover similar to those exposed to E2 and shared common signalling pathways for E2 and FLX in their proteome. These results confirm the endocrine disrupting potential of antidepressants with respect to sex steroid signalling in vertebrates.

The importance of neuroendocrine signalling renders invertebrate endocrine systems preferential targets for SSRIs. Indeed, FLX stimulated the synthesis and release of crustacean hyperglycaemic hormone and the mobilisation of glucose, leading to increased locomotor and burying activity in the European shore crab *Carcinus maenas*. Furthermore, FLX stimulated the release of moult inhibiting hormone resulting in significantly lowered 20-hydroxyecdysone levels indicative of endocrine disruption of moulting. Exposure of juvenile shore crabs to a mixture of FLX and venlafaxine (VEN) significantly reduced the animal's colour change capacity, putatively by stimulating pigment dispersing hormone, thereby compromising camouflage. In

the European cuttlefish, *Sepia officinalis*, mixtures of FLX and VEN negatively affected the maturation of feeding and burying behaviour in newly hatched animals over the first 5 and 30 days, respectively. The changes in predatory behaviour tended to reduce food intake and significantly affected growth after 30 days of exposure to FLX+VEN. On the contrary, matching to uniform backgrounds was improved without affecting disruptive body patterns. The results provide indirect evidence for antidepressant derived modulation of neuronal and neuroendocrine signalling in crustacean and mollusc by altering their neurotransmitter levels.

### **1.13.P-Tu048 In Silico Profiling of Triclosan and its Metabolites as Reproductive Toxic Agents**

*Maja Milanović, Nataša Milošević, Larisa Đurić, Jovana Drljača Lero and Nataša Milić, Department of Pharmacy, University of Novi Sad, Faculty of Medicine, Serbia*

More than thousands of chemicals are associated with endocrine disruption. However, the data about the association between the exposure to an endocrine disrupting chemical and fertility are mostly incomplete. Triclosan is a halogenated biphenyl ether widely used in different cosmetic products, disinfectants, clothing textiles, and other materials. The high production rates and excessive use of triclosan contribute to the long-term exposure via air, surface water, drinking water, and soil. Hence, triclosan ability to impair the reproductive function through multiple nuclear receptors raises concerns. The aim of this study was to evaluate the potential of triclosan and its metabolites to interfere with estrogen receptors using molecular docking approach. The affinity of triclosan, triclocarban, methyl-triclosan and monohydroxy-triclosan derivatives for estrogen receptor alpha (ERalpha; PDB entry code: 6VPF), beta (ERbeta; PDB entry code: 5TOA), and gamma (ERgamma; PDB entry code: 6KNR) was determined using Genetic Optimisation for Ligand Docking (GOLD) version 2020.3.0. Bisphenol A was used as reference ligand. ChemPLP fitness scores were applied for the evaluation of binding affinities of the analysed compounds. Molecular properties were predicted using SwissADME online tool. Based on the obtained results all examined compounds have desirable properties in the terms of physicochemical characteristics expected for optimal oral bioavailability. The affinity scores for ERalpha receptor were from 50.4 to 57.9. The obtained ChemPLP scores of triclosan and its metabolites for ERbeta and ER gamma receptors were in the same range (41.3-56.9 and 53.5-58.4, respectively). Monohydroxy-triclosan metabolites expressed the highest score for ERalpha and ERbeta while methyl-triclosan showed the highest affinity for ERgamma. In compared with bisphenol A as reference compound (ERalpha-64.5, ERbeta-51.9, ERgamma-57.2) triclosan and its metabolites demonstrated the similar affinity to estrogen receptors. Based on the obtained results triclosan and its metabolites might act as agonist or antagonist on estrogen receptors. Therefore, the potential role of triclosan as risk factor for the development of infertility should be further examined.

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### **1.13.P-Tu049 Endocrine Disrupting Potential of Beta-Blockers Exposure - Computational Study**

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$\beta$ -blockers are the class of pharmaceuticals frequently prescribed for treatment of cardiovascular disorders. Owing to the high consumption and physicochemical properties,  $\beta$ -blockers undergo wastewater treatment process. Hence, they can migrate through surface water and reach the groundwater and, ultimately, the drinking water. Therefore, the  $\beta$ -blockers effects on the human endocrine system and its function arouses serious concerns among scientists since their negative impact on aquatic organisms has been well documented. The aim of this study was to investigate  $\beta$ -blockers' potential to interrupt the human endocrine system using molecular docking. Propranolol and 16 structurally related compounds were selected using DrugBank base. The interaction between analysed  $\beta$ -blockers and estrogen receptor alpha (ER $\alpha$ ; PDB entry code: 7UJ8) and androgen receptor (AR; PDB entry code: 5CJ6) was determined using GOLD molecular docking tool. Docked poses were scored using ChemPLP function. All  $\beta$ -blockers expressed the high affinity to estrogen and androgen receptors. The binding affinities of the analysed compounds for ER $\alpha$  were in the range 51.4-87.3 (co-crystallized 4-Hydroxytamoxifen affinity-96.8). Carvedilol, nebivolol and bisoprolol showed the highest ChemPLP score. Dexpropranolol, nadolol and propranolol demonstrated the higher affinity towards androgen receptor in compared with the co-crystallized ligand (2-Chloro-4-[(2-hydroxy-2-methyl-cyclopentyl)amino]-3-methyl-benzonitrile). The evaluated scores of the analysed compounds for AR were in the range 46.8-77.5 (referent ligand score-68.4). The binding affinity of  $\beta$ -blockers is governed by at least one chiral center and the presence of an aromatic or heteroaromatic ring with an aryloxypropanolamine side chain. Based on the obtained results  $\beta$ -blockers have ability to disrupt endocrine function by binding to both estrogen and androgen receptors. Further studies are needed in order to fill the knowledge gaps about the adverse effects of  $\beta$ -blockers present in the environment on human health.

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## **1.14 Unravelling the Complexities of PFAS: From Environment to Human Health and Reproduction**

### **1.14.T-01 Evaluation of PFAS Effects on Nuclear Receptors and Cellular Pathways Using High-throughput Screening Datasets**

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Per- and polyfluoroalkyl substances (PFAS), a group of man-made synthetic organofluorine chemicals containing a carbon-fluorine bond, are widely used in consumer products around the world. PFAS are also called “Forever Chemicals” because their persistence has led to extensive environmental contamination and bioaccumulation. The combination of wide use and poor degradation of PFAS raises large public health concerns. To quickly assess PFAS, especially those not well-studied, for their potential toxicological effects and mechanisms, the Tox21 in vitro assay datasets generated from screening thousands of environmental chemicals against various cellular targets and pathways were extensively analyzed. This presentation will provide an overview of a panel of the in vitro assays used for the Tox21 high-throughput screening, summarize the findings of a group of PFAS for their activities on nuclear receptors and cellular signaling pathways, and compare with the data generated from other in vitro high-throughput screening platform.

#### 1.14.T-02 In silico and in vitro prioritization of emerging PFASs identified by non-targeted analysis for environmental hazard assessment

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Per- and polyfluoroalkyl substances (PFASs) has raised great environmental concerns due to their persistence, bioaccumulation and toxicity. The restricted use of classical PFASs has led to the development of alternative PFASs. Unauthorized release of “new” PFASs that have not been through hazard assessment can also pose risks to human and environment. Through non-targeted analysis, close to one hundred emerging PFASs were identified in the surface water near an industrial park in China. How to efficiently assess the ecological hazards of the emerging PFASs with strong laboratory animal welfare considerations needs to be quickly resolved. In this study, we have developed a tiered workflow, mainly utilizing *in silico* and *in vivo* new approach methodologies (NAMs), to prioritize key emerging PFASs from a large list of non-targeted screening for in-depth hazard assessment. According to the concentrations in river and structural differences of emerging PFASs, eight PFASs were selected for QSAR analysis, molecule docking, and in vitro high-throughput screening (zebrafish hepatic cell-line). PFOA was used as a reference compound along with each assay. The results showed that the predicted acute toxicity of HFPO-TA and HFPO-TEA was found to be significantly higher than that of PFOA, warranting follow-up assessment. 6:2 FTCA has the same carbon chain length with PFOA, however, the predicted toxicity of this emerging PFAS is significantly lower compared to PFOA, providing novel insights into future design of alternative. The in vitro results also indicated that neither HFPO-TA nor HFPO-TeA are suitable substitutes for PFOA, emphasizing the importance of assessing their environmental hazards. The workflow developed in this study will not only facilitate cost-efficient assessment of new PFASs, but will also shed light on next generation hazard assessment of other emerging contaminants using non-animal methods.

#### 1.14.T-03 The Relationship Between the Molecular Properties of Per- and Polyfluorinated Substances (PFAS) and Their Ecotoxicity

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Per- and polyfluorinated substances (PFAS) are a highly diverse group of very persistent and bioaccumulative compounds. Currently, ecotoxicity data are available for only a limited subset of PFAS, such as PFCAs and PFASAs, which poorly represent the plethora of known PFAS molecular structures. Since it is not feasible to test all PFAS for their ecotoxicity, a more viable approach would be to identify potential relationships between ecotoxicity and molecular properties, allowing to predict PFAS ecotoxicity based on molecular structures. Therefore, this study aimed to relate the ecotoxicity of 13 PFAS varying either in the fluorocarbon chain length (7 PFCAs) or in the polar head group (6 PFAS with 4 fluorocarbons), to their membrane-water partition coefficients ( $K_{MW}$ ), a relevant molecular descriptor. We determined the acute ecotoxicity of the selected PFAS to the daphnid *Daphnia magna*, and the chronic ecotoxicity to the green alga *Raphidocelis subcapitata* and the cyanobacterium *Synechocystis* sp. PC6803. The narcotic  $LC_{50}$  calculated using the  $K_{MW}$  and the critical body burden very well predicted the ecotoxicity of PFCAs to *R. subcapitata* and *D. magna*, which increased with increasing fluorocarbon chain length. *Synechocystis* displayed a different response pattern, with  $EC_{50}$  values increasing as the length of the fluorocarbon chain increased from four to six carbons, followed by a decrease from six to eight carbons. For the 4-fluorocarbon PFAS the polar head group greatly impacted ecotoxicity, with  $EC_{50}$  values varying up to three orders of magnitude. Most toxic to all tested organisms was perfluorobutane sulfonamide (FBSA,  $C_4F_9-SO_2NH_2$ ), with  $EC_{50}$  values of 33.9 mg L<sup>-1</sup> for *D. magna*, 18.5 mg L<sup>-1</sup> for *R. subcapitata* and 18.5 mg L<sup>-1</sup> for *Synechocystis*. Perfluoropentanoyl chloride (PFPeCl,  $C_4F_9-CClO$ ) was the least toxic to all test organisms, with  $EC_{50}$  values of 10850 mg L<sup>-1</sup> for *D. magna*, 819 mg L<sup>-1</sup> for *R. subcapitata* and 3362 mg L<sup>-1</sup> for *Synechocystis*. This could be attributed to the differences in charge distribution around the molecules resulting from distinct polar head groups. These differences may affect the affinity of the PFAS to the phospholipid bilayer of cell membranes, subsequently influencing their ability to protrude into the membrane. Our findings indicate that  $K_{MW}$  may be a good molecular predictor of PFAS ecotoxicity, and highlight the significance of expanding ecotoxicity studies to a broader range of structurally diverse PFAS.

#### **1.14.T-04 Integrating Experimental and Computational Approaches to Unravel the Toxicity of 33 PFAS Congeners in Human Cell Lines**

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Per- and polyfluoroalkyl substances (PFAS) present complex challenges in toxicology, prompting a multidimensional exploration of their impact. In this study, we employed a holistic approach to investigate the cellular toxicity and nuclear receptor activation of 33 PFAS congeners, spanning legacy and next-generation compounds within the (poly)ether carboxylic or sulphonic groups.

Three human cell lines—HepaRG (liver), HEK293T (kidney), and A549 (lung) and human Peripheral blood mononuclear cells—were subjected to varying exposure concentrations, albumin concentrations, ionic strength of the medium, exposure durations, and assay types. Data from both experimental and computational analyses were seamlessly integrated to model toxicity and nuclear receptor activation. This integrative approach not only provides a quantitative framework for understanding PFAS toxicity but also facilitates predictive modeling for risk assessments. For a comprehensive computational analysis, descriptors were generated using the CDK library. Among these, concentration, VP7 (a measure of a molecule's size), fragC (a measure of complexity based on constituent fragments), MDEC34 (a measure of size and branching), and VPC4 (a measure of size and shape) emerged as the most influential features for cellular toxicity.

Simultaneously, we developed a Quantitative Structure-Activity Relationship (QSAR) model also for predicting PPAR $\alpha$  induction. The model utilized dose-response data obtained with HEK293T cells, binding energy estimates from molecular dynamics simulations, and 133 computational descriptors generated through the CDK library for feature selection. The most important features identified for the QSAR model include concentration, Weta3.unity (a measure of structural complexity), binding energy, VP7 (a measure of a molecule's size), and ECCEN (a measure of connectivity and symmetry).

Our study unveils a nuanced perspective on the diverse toxicological profiles of PFAS congeners, considering both experimental intricacies and computational insights. These findings hold promise for advancing risk assessment methodologies and tailoring mitigation strategies to address the complexity of PFAS toxicity.

#### **1.14.T-05 Characterization of Replacement PFAS, Perfluoroethylcyclohexane Sulphonate (PFECHS) and Perfluorobutane Sulphamide (FBSA) in vitro Individually and in Mixture with PFOS**

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The widespread application of poly- and per-fluoroalkyl substances (PFAS) has resulted in some substances being ubiquitous in environmental matrices with associated toxic effects. While certain substances of concern have been phased-out or banned, new substances continue to be produced. Two such substances are perfluoroethylcyclohexane sulphonate (PFECHS) and perfluorobutane sulphamide (FBSA), both of which are developed analogues and replacements of perfluorooctanesulphonic acid (PFOS) which have recently been detected in multiple environmental media around the globe. However, there is little information on the toxic potency of PFECHS and FBSA relative to legacy substances and other known PFAS replacements. As well, although it is more environmentally relevant, there is little data available outlining the effects of PFAS mixtures, particularly when it comes to mixtures of PFAS replacements. Therefore, this research investigated whether binary mixtures of emerging and legacy PFAS have the potential to combine synergistically. The immortalized rainbow trout gill cell line (RTgill-W1) was chosen as the experimental model to investigate two apical endpoints: cytotoxicity and lipid toxicity. RTgill-W1 cells were exposed for 24 hours to each compound from a range of 1 to 1000 mg/L to obtain endpoint and compound specific lethality and effect concentrations (LC<sub>x</sub>;EC<sub>x</sub>). These values were then applied to formulate mixture predictions following the Loewes Additivity and Steel and Peckham methods. Four mixture combinations involving five exposure ratios were investigated: PFECHS-PFOS, FSBA-PFOS, FSBA-PFECHS for cytotoxicity, and PFECHS-PFOS for lipid toxicity. Individual compound potency ratios were as followed: PFOS > PFECHS > FSBA for cytotoxicity. PFOS and PFECHS had a nearly identical impact on phospholipidosis, while FSBA did not have any impact. Most mixtures had a synergistic effect on cytotoxicity, but the effect was both dose and ratio dependant. However, PFECHS and FSBA mixtures were synergistic at all combinations. For phospholipidosis, the PFECHS – PFOS mixture was strictly additive at all combinations. These results are suggestive of synergism between emerging PFAS replacements and highlight that independent apical mechanisms of different PFAS could combine to induce unexpected toxicity. Considering that emerging replacements are continuing to increase in concentration in the environment, such mixture scenarios are likely to increase in probability.

#### **1.14.P Unravelling the Complexities of PFAS: From Environment to Human Health and Reproduction**



#### 1.14.P-Tu051 Predicting Peroxisome Proliferator-Activated Receptors- $\gamma$ Cytotoxicity of Small Molecules: A Synergistic Consensus Model and Deep Learning Binding Affinity Approach powered by Enalos Cloud Platform

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The Enalos Cloud Platform, accessible at <https://www.enaloscloud.novamechanics.com>, hosts a synergistic consensus model for predicting the Peroxisome Proliferator-Activated Receptors (PPAR)- $\gamma$  cytotoxicity of small molecules. This in silico model was developed using a comprehensive dataset of 6,538 compounds from the Tox21 10K chemical library, sourced from PubChem Bioassay ID 743194 with Enalos tools (<https://enalosnodes.novamechanics.com>). These compounds were screened against the PPAR $\gamma$ -bla HEK293H cell line and categorized based on cell viability.

Developed with the aid of the Isalos Analytics Platform (<http://isalos.novamechanics.com/>), the model integrates three distinct modeling methods:

- A Random Forest model, employing an ensemble learning method that combines multiple decision trees to enhance predictive accuracy.
- A k-Nearest Neighbors (k-NN) model, also known as read across, which offers a straightforward yet effective non-parametric technique for managing a wide range of data types.
- A binary class Support Vector Machine (SVM) model, utilizing Stochastic Gradient Descent for hinge loss minimization and producing a robust, margin-based classifier that optimizes class separation.

These methods are integrated with molecular descriptors obtained from the molecules' 2D structures, using the EnalosMold2 node. The model interfaces with a deep learning model via Enalos Application Programming Interfaces (APIs), boosting the prediction of small molecule binding affinity to PPAR $\gamma$ . It leverages the deep learning capabilities of the Enalos Cloud for structure-based information integration. Inputs for the model include SMILES notation, SDF files, or use of a molecular sketcher, and it outputs a classification of 'Active' or 'Inactive' for each compound. The model also assesses prediction reliability based on the Applicability Domain, ensuring predictions stay within the training data's scope. By synergistically blending molecular docking, machine learning, and deep learning techniques with chemoinformatics, the consensus model emerges as an advanced tool for predicting PPAR $\gamma$  cytotoxicity, with a focus on per- and polyfluoroalkyl substances (PFAS). This positions it as an invaluable asset in drug discovery and toxicology. The model is accessible via a user-friendly graphical user interface (GUI) at <http://www.enaloscloud.novamechanics.com/scenarios/ppargammaliganddl/>, providing a cutting-edge tool for scientific and biomedical research globally.

#### 1.14.P-Tu052 Assessing Apical Toxicity and Sublethal Responses of Earthworms in PFAS-Contaminated Soils: A Comprehensive Study at a Fire Training Site in Sweden

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Fire training sites and airports are major PFAS contamination sources due to widespread Aqueous Film-Forming Foam (AFFF) use. Recent global policies restrict higher than C8 PFAS congeners, yet PFOS and PFOA legacy persists, impacting ecosystems. Modern AFFF formulations still include short-chain PFAS and their oxidizable fluorolalkylamide precursors. Terrestrial ecosystems, vital for ecosystem services, face threats. Earthworms, essential bioindicators, play a key role in soil processes. This study assesses PFAS-contaminated AFFF impact on earthworms, highlighting ecological consequences and the need for sustainable firefighting policies.

The study investigated apical toxicity and sublethal responses of earthworms across a PFAS contamination gradient from a Trelleborg drill site. PFAS contamination was confirmed, with 22 compounds, mainly PFOS, PFOA, PFNA, and PFHxS, ranging from 960 ppb (B1) to 8.7 ppb (B7). OECD No. 207 (acute toxicity) and No. 222 (reproduction) tests, plus 30-day sublethal assessments, were conducted. Biomarkers included oxidative burst, enzymatic responses, and an escape test. Pristine soil served as an external reference control.

Acute toxicity, seen in B1 and B4, indicated a significant 20% and 12.5% mortality increase (n=4), OECD No. 207. Reproduction tests are ongoing. Immunological responses showed higher reactive oxygen-producing cells in B1 and B4, with lower phenol oxidase in PFAS-impacted sites. Catalase increased in Trelleborg sites, spiking in B6 (22 PFAS 9.6 ppb). Acetylcholinesterase fluctuated, reducing in B4 and linked to impaired behavior as judged in an escape time, showing PFAS sensitivity. Further neurotransmitter investigations are ongoing to unveil potential neurotoxicity.

These findings contribute significantly to the broader understanding of PFAS-contaminated site ecology, aiding in the construction of an environmental database crucial for risk assessment analyses. The call for sustainable firefighting practices is not just a regulatory necessity but a vital step towards preserving terrestrial ecosystems and the invaluable services they provide.

#### **1.14.P-Tu053 Investigating the impact of PFOA, Glyphosate and Cypermethrin on the gut microbiota in vitro**

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The human gut microbiota is the totality of the microorganisms that colonizes our gut and many studies have demonstrated its involvement in human health and in the development of many diseases. Humans get in contact with hundreds of chemicals every day, thus the gut microbiota is daily exposed to many factors that could alter its status. The aim of this study was to investigate, using an *in vitro* approach, how three different commonly used chemicals PFOA, a forever chemical, Glyphosate, a widely used and controversial herbicide and Cypermethrin, a synthetic pyrethroid pesticide highly toxic to fish, bees and aquatic insects, affect the gut microbiota.

We implemented and validated an *in vitro* method for the culture of gut microbiota that maintains the functional and compositional profiles of *in vivo* gut microbiota. Then, stool samples from healthy donors were employed to test the impact of chemicals on the gut microbiota using metaproteomic and metabolomic analyses. The *in vitro* gut microbiota models were treated separately with PFOA, Glyphosate and Cypermethrin at different concentrations. Control samples grown without chemicals were also prepared and analyzed.

The metaproteomic analyses of the *in vitro* experiments were used to perform both phylogenetic and functional analysis. At the level of phyla only the stimulation with Glyphosate led to statistically significant changes. For the other taxonomic levels investigated -orders, genera and species- all the chemicals led to significant alterations. Almost all modulated taxa were previously studied either to be important for the health and stability of the gut (decreased levels after treatments) or involved in some inflammatory processes (increased levels after treatments). The functional analysis of the samples treated with the higher concentration of Glyphosate showed a clear distinct pattern, at the molecular functions and biological processes levels, in respect with the control group.

Glyphosate, PFOA and Cypermethrin strongly altered the gut microbiota *in vitro*, suggesting a potential direct association with gut microbiota dysbiosis mechanisms, but further studies are needed. The *in vitro* approach coupled with metaproteomic and metabolomic analysis is a valuable tool to investigate the impact of chemicals on the gut microbiota.

#### **1.14.P-Tu054 Unraveling the Proteome Landscape of Perfluorooctane Sulfate Exposure During Pathogen-Associated Molecular Pattern Challenge in Peripheral Blood Mononuclear Cells**

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An increasing body of evidence suggests that per- and polyfluoroalkyl substances (PFAS) may adversely affect the immune system, potentially leading to immune dysregulation, decreased vaccination response and increased susceptibility to infections. In this study, we delve into the intricate molecular changes occurring within peripheral blood mononuclear cells when exposed to perfluorooctane sulfate (PFOS) during a lipopolysaccharide (LPS) challenge, a well-established pathogen-associated molecular pattern. PFOS is a persistent environmental contaminant with known health implications, and its interaction with immune cells remains largely undisclosed. By employing advanced proteomic techniques, we aim to unravel the complex molecular landscape, shedding light on the cellular responses and potential biomarkers associated with PFOS exposure during an inflammatory challenge with LPS as a representative PAMP.

To this end, PBMCs were pre-treated *ex vivo* with increasing amounts (3-600 µM) of PFOS for 24 hours, followed by LPS challenge after an additional 12 hours. Proteomic analysis was carried out on nano chromatography coupled to high resolution mass spectrometry using a 40-minute gradient with a Data Independent Analysis approach. Data were analyzed using DIA-NN software.

More than 4200 proteins were quantified across all the samples. The results showed that PFOS strongly impacted the PBMC proteome both at low and high concentrations. The main pathways altered by the PFOS treatments were related to

inflammatory and immune responses. In particular, the treatment at 3 microM showed a marked increase of proteins associated with innate immune response (IFIH1, IFIT2, DAPK1, MX1, CASP4, ISG20, IN35, etc.), defense immune response (CD48, STAT4, LIRA1, etc.), cytokine-mediated signaling pathway (IL1A, IL1B, LIRB4, among others.), while at 600 microM we found an enrichment of inflammatory response (IL6, IL8, CXCL5, CXCL2, etc.), interleukin 8 and 2 production, and a positive regulation of NF- $\kappa$ B transcription factor (KPCD2, TRI22, TRAF1) proteins. These pathways are essential for inflammatory response mechanisms.

Our findings provide valuable insights into the intricate interplay between PFOS exposure and immune and inflammatory responses, contributing to a deeper understanding of the health implications associated with environmental PFOS exposure.

#### **1.14.P-Tu055 Chronic effects of Perfluorooctane Sulfonic acid (PFOS) on *Cloeon dipterum* larvae development**

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**Chronic effects of Perfluorooctane Sulfonic acid (PFOS) on *Cloeon dipterum* larvae development**

Perfluorooctane sulfonic acid (PFOS) is of high concern because of its widespread application in consumer products and its toxic effects on human and animal health. PFOS has been known to be extremely environmentally persistent and has bioaccumulative toxic effects. Previous studies assessed the acute effects of PFOS on the traditional endpoints mortality and immobilizations. However, we evaluated the long-term exposure of PFOS effects on the aquatic *Cloeon dipterum* larvae development, survival, and emergence. Third- and fourth-instar larvae were exposed to five concentrations ranging from 0 to 100  $\mu$ g/L. The test duration was 31 days. The renewal was done after every two weeks. Animals were fed on commercial snail food every 3 days. During the experiment survival, larvae development, larvae growth, and emergence were evaluated.

Our results show reduced larvae development, greater larvae mortality, and less emergence within the PFOS treatments compared to control. Larvae development was the least sensitive endpoint (NOEC = 10 $\mu$ g/L). Moreover, significant delays in emergence were observed in all the treatments compared to the control. Emergence was relatively less in the highest treatment 100 $\mu$ g/L. The proportion of larvae that survived until emergence did not significantly differ between the control and treatments up to 10  $\mu$ g/L after 31 days. The present study indicates that aquatic insects appear to vary greatly in their sensitivity to PFOS depending on endpoints. This might be explained by differences in the physiological responses to PFOS and the toxicokinetics of PFOS. Based on these results follow-up experiments for PFOS toxicokinetics and toxicodynamics in insects will be conducted in 2024.

#### **1.14.P-Tu056 Significant Changes Induced By PFOA And GenX Environmental Concentrations In Guppy Testes Transcriptome And Reproductive Traits**

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In humans and rodents, exposure to PFAS has been frequently linked to adverse reproductive health outcomes, but our knowledge of their effects on reproduction remains limited in aquatic organisms. To help filling this gap, we exposed adult males of a freshwater fish (*Poecilia reticulata*) to 1 mg/L of PFOA, a legacy PFAS, or its alternative, GenX, potentially less hazardous.

After 21 days of exposure, we assessed PFAS bioaccumulation in whole-body fish and the expression of the reproductive traits associated with male mating and insemination success, such as body colouration, sexual behaviour (number of sigmoid displays and gonopodial thrusts), ejaculate quality traits (sperm swimming velocity, number, and viability). Then, we performed a transcriptomic analysis (RNA-seq) in testes, a key tissue for the study of the effects on male reproduction.

PFOA exhibited higher bioaccumulation than GenX. Although overall sexual activity was not significantly affected by treatment, GenX (but not PFOA) males performed more courtship displays than controls. As behavioural tests were conducted with unreceptive females, this result can be seen as maladaptation. As to ejaculate traits, both GenX and PFOA reduced sperm swimming velocity compared to controls. This could be attributable to PFAS entering the testes and causing direct detrimental effects on mature sperm, or to PFAS impairing the molecular mechanisms regulating sperm maturation. Accordingly, the transcriptional modulation of some genes contributing to sperm velocity seems to support the observed decrease in sperm velocity, thus linking molecular and phenotypical findings. Overall, compared to controls, we identified a total of 86 and 107 differentially expressed genes in GenX- and PFOA-treated fish, respectively. Modulated genes were involved in immune response, spermatogenesis, antioxidant activity, sperm quality, and lipid metabolism.

In conclusion, we showed that reproductive traits and gene expression were sensitive endpoints highlighting PFAS toxicity at low - environmentally relevant - concentrations in male guppies. The detrimental effects we demonstrated are of great relevance, especially considering that the environmental exposure to alternative PFAS will grow in the future. Our results provide important evidence suggesting that the toxicity potential of GenX may be higher than that of PFOA, despite the fact that GenX is deemed less toxic, at least in the species used here.

**1.14.P-Tu057 Assessment of Per and Polyfluorinated Alkyl Substances (PFASs) Residues in Urine samples of children**  
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Children are a part of the population that is particularly susceptible to toxicants and, especially, to those that can have a harmful impact on their health. In this context, we wanted to investigate the exposure of early stage population to commonly known per- and polyfluoroalkyl substances as well as any other new PFAS used as a substitute. For this reason, a suspect screening methodology was developed for the study of 40 per- and polyfluoroalkyl substances including the banned PFOA and PFOS, among other 18 legacy PFASs, and the ones considered as new replacement PFASs such as the short TFA, ADONA, and GenX, among others, in urine as a non-invasive matrix. This study includes 185 urine samples from 8-year-old children Inma Asturias cohort.

The samples were analyzed with a liquid chromatography coupled to high resolution mass spectrometer. The separation of legacy PFASs was achieved with a Hypersil Gold PFP column while the short PFASs were separated in an Atlantis Premier BEH C18 AX column.

The main results showed the presence of legacy carboxylic acid PFASs such as PFPeA, PFHpA, PFOA, PFNA, PFUdA and the sulfonates PFHxS, PFOS and PFNS, at concentrations ranging from 4.9 ng/L (PFNS) to 532 ng/L (PFHxS). In addition, some short chain PFASs such as TFA have been detected in some punctual samples. The two most frequently detected compounds have been the short TFA and GenX being present in 51% and 50% of the samples, respectively, at quantifiable concentrations.

The values detected in this work were comparable to the ones reported for children but at much lower concentration compared with the ones reported in the literature for adult people and workers from a PFASs manufacture. However, it is important to remark that this study has been focused on 8-years-old children so their exposure to these toxicants has been basically through the diet and, therefore, these concentrations could increase through the years since their exposure could be chronically

**1.14.P-Tu058 Bioaccumulation and Ecotoxicity of Representative PFAS in Model Marine / Estuarine Species**

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In coastal marine/estuarine areas, PFAS concentrations in sediments have typically been reported in the ppb range (i.e., 0.1 to 10 ng/g or ppb), Salinity influences the fate and transport of PFAS (i.e., inverse conc. relationship in seawater with increasing salinity) and sediments appear to serve as a repository (i.e., shorter chain length PFAS primarily associated with sediment porewater while longer chain length PFAS bound to sediment). Currently there is not sufficient data to derive toxicity reference values (TRVs) and/or quantify uptake bioavailability in marine species. This study will quantify the bioavailability and toxicity of 7 PFAS related to legacy aqueous film forming foams (AFFF) in acute and chronic tests with 10 model marine species representing benthic, epibenthic and pelagic species. The 7 PFAS include PFBA, PFHxS, PFHxA, PFOA, PFOS, 6:2 FTS and 8:2FTS. Benthic species will be exposed to spiked sediments while epibenthic and pelagic species will be evaluated in aqueous exposures. In addition to toxicity endpoints (LC50, EC50, NOEC, and LOEC values), bioaccumulation factors (BAFs) and Bioconcentration Factors (BCFs) will be derived for select species (i.e., those with sufficient tissue mass) in sediment and aqueous exposures, respectively. Results of studies for sediment spiking procedures demonstrate complete transference of PFAS sorbed to sand onto sediment particles. A high percentage (>75%) of spiked PFOS, PFNA and 8:2 FTS was associated with sediment particles while PFHxA, PFHxS and PFOA was mostly (>75%) associated with sediment porewater in spiked sediments. Preliminary aqueous acute toxicity results with the silverside minnow (*Menidia berylina*), the sheepshead minnow (*Cyprinodon variegatus*), the grass shrimp (*Palaemonetes pugio*), the mysid shrimp (*Americamysis*), the sea urchin (*Arbacia punctulata*) and the copepod (*Acartia*), show PFASs to be more toxic than PFCAs or the Fluorotelomers evaluated. Of the species evaluated *Acartia* is the most sensitive followed by *Americamysis*. Testing is ongoing and results will be presented as available.

**1.14.P-Tu059 Bioaccumulation and Toxicity of Field-collected PFAS-impacted Sediment to *Leptocheirus plumulosus* and *Chironomus dilutus***

*Guilherme R. Lotufo, Paige M Krupa, David W. Moore and Ashley N Kimble, U.S. Army Engineer Research and Development Center*

Per- and polyfluoroalkyl substances (PFAS) are currently the focus of much attention due to their ubiquitous occurrence, environmental persistence, and implications to human and ecological health. However, data gaps exist regarding the fate and effects of these substances in aquatic environments, particularly for estuarine and marine systems. Most aquatic toxicity and bioaccumulation studies to date focus on water-column exposures, with less data available for sediment-based exposures. Furthermore, few studies describe effects of sediment-based PFAS at environmentally relevant concentrations. To address this knowledge gap, the current study collected sediment from 16 estuarine and 10 freshwater sites suspected of having elevated levels of PFAS from various sources. Estuarine sediments were evaluated using 28-d bioaccumulation and 10-d toxicity tests with the amphipod *Leptocheirus plumulosus* while freshwater sediments used 16-d midge (*Chironomus dilutus*) bioaccumulation and toxicity tests. Measured  $\Sigma$ PFAS in sediment ranged from 1 to 120 ng/g ww, with long-chain compounds

accounting for the majority of total PFAS in both estuarine and freshwater sediments. Survival and growth of organisms in the toxicity tests did not appear to be affected by the PFAS-impacted sediment. Preliminary tissue data for *L. plumosus* suggests that biota-sediment accumulation factors (BSAFs) varied between the different PFAS analytes and sites. Additionally, preliminary data did not indicate a clear or consistent trend between bioaccumulation and carbon chain length, percent organic matter, or sand/fine sediment type. As the differential bioaccumulation observed was likely influenced by physical and chemical properties of the sediment, subsequent data analysis will more look into the role of various sediment properties upon bioaccumulation.

#### **1.14.P-Tu060 Biomagnification Or Per/Polyfluoroalkyl Substances Through Mixture Uptake from Soil in a Terrestrial Food-Web**

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We are determining biomagnification of PFAS in a terrestrial food-web from lower trophic level organisms to higher trophic level organisms using environmentally relevant mixtures and concentrations. We quantified the uptake of these PFAS in terrestrial plants from soil and generated bioconcentration factors (BCF). Based on the results of these investigations, we selected PFAS compounds with BCF values of two or greater for the biomagnification study. This study is determining biomagnification calculated as Trophic Transfer Coefficients (TTC) for the chemical transfer of individual PFAS from terrestrial plants grown in PFAS amended soil to mammals (Dutch belted rabbits), exposed to PFAS through their daily diet. Results to date contributed to further refinement of what PFAS, what tissues, what organisms or what trophic paths may be more risk relevant for the variety of sites and scenarios within the interests of PFAS ecotoxicology at large. Ecologically relevant exposure data developed in this research will fill several knowledge gaps regarding biomagnification potential for PFAS compounds released into the terrestrial environment.

#### **1.14.P-Tu061 The Transgenerational Adverse Effects Caused by GenX on Locomotive Behaviors in *Caenorhabditis elegans***

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Per- and polyfluoroalkyl substances (PFASs), with nearly 15000 variants produced today, exhibit widespread use in industrial and consumer products owing to their amphipathic nature, as well as their chemical and thermal stabilities. Concerns have emerged due to the adverse health effects associated with human exposure to PFASs, prompting the search for substitutes. For instance, perfluorooctanoic acid, one of the most commonly used PFASs, has been linked to adverse effects such as reduced reproduction and impaired locomotion in zebrafish. Moreover, it raises the possibility of transgenerational transmission, which is known as the "transgenerational effect".

To reduce concerns caused by perfluorooctanoic acid, GenX emerged as a substitute with a shorter carbon chain length, anticipated to be less toxic. However, studies have indicated that GenX may still induce toxic effects such as hepatic or reproductive toxicities. As current research primarily focuses on these aspects, there are gaps in comprehending the neurotoxic effects and their underlying mechanisms. Additionally, whether GenX can induce transgenerational effects remains unclear. Under these circumstances, our study aims to investigate the transgenerational neurotoxic effects induced by GenX.

This study focuses on understanding the GenX induced transgenerational neurotoxic effects in the *Caenorhabditis elegans* model, as well as elucidating the potential underlying mechanisms. Our preliminary findings indicate that GenX exposure at various concentrations (0, 0.0003, 1, 10, 100, 1000, 10000, and 30000  $\mu\text{M}$ ) results in inhibited growth (10000 & 30000  $\mu\text{M}$ ), reproduction (0.0003-10000  $\mu\text{M}$ ), and locomotive behaviors including body bend and head thrash (0.0003-10000  $\mu\text{M}$ ). Moreover, we found that the phenomenon of the decrease in locomotion can be transmitted from parental generation to their offspring (F1-F3) under 0.0003  $\mu\text{M}$  exposure, suggesting that GenX could cause the transgenerational neurotoxic effects and may not be safer than we thought.

The significance of this research lies in advancing our understanding of the potential transgenerational neurotoxicity associated with GenX exposure and its implications for human health. This study contributes essential insights to the broader discussion on PFAS substitutes and their implications for human health.

#### **1.14.P-Tu062 Insight into the differential toxicity of PFOA and PFBA based on a 3D-cultured MDA-MB-231 cell model**

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Perfluoroalkyl substances (PFASs) are a category of high-concerned emerging contaminants which are suspected to correlate with various human adverse health outcomes including tumors. It is also a question whether short-chain PFASs are qualified alternatives under the regulation of long-chain PFASs. In this study, a three-dimensional (3D) culture system based on Gelatin methacrylate (GelMA) hydrogel matrix was used to investigate the impacts of 120-h perfluorooctanoic acid (PFOA) and perfluorobutanoic acid (PFBA) exposure of MDA-MB-231 cells. The results showed that PFOA exposure promoted the proliferation, migration, and invasion of MDA-MB-231 cells in an environmentally relevant concentration range (0.1 to 10  $\mu\text{M}$ ), exhibiting a clear malignant-promoting risk. In contrast, PFBA only showed a trend to induce non-invasive cell

migration. Hippo/YAP signaling pathway was identified as the contributor to the differences between the two PFASs. PFOA but PFBA reduced YAP phosphorylation and increased the nuclear content of YAP, which further facilitated abundant key factors of epithelial-mesenchymal transition (EMT) process. Our results provided a new idea for the carcinogenicity of PFOA using a 3D-based paradigm. Although the effects by PFBA were much milder than PFOA in the current test duration, the cell model suitable for longer exposure is still necessary to better assess the safety of alternative short-chain PFASs.

#### **1.14.P-Tu063 Environmental Modeling Of Trifluoroacetic Acid (TFA) Originating From Hydrofluoro-Olefins**

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Hydrofluoro-olefin HFO-1234yf (2,3,3,3-tetrafluoropropene), the key mobile air conditioning refrigerant, degrades in the atmospheric environment to trifluoroacetyl fluoride which then hydrolyses to form trifluoroacetic acid (TFA). The high-water solubility of TFA together with its ability to spread rapidly in the environment via the water cycle makes it widespread and ubiquitous in nature. This modelling study assessed the fate and transport of TFA in the freshwater aquatic environment resulting from the emission and atmospheric degradation of HFO-1234yf and subsequent atmospheric deposition along the Rhine River basin.

The Rhine was selected as a case study as it represents a prominent watershed in Western Europe with a large population. Environmental fate and transport modelling of TFA in the freshwater aquatic environment was conducted based on the assumption of constant and ongoing HFO emissions, which through atmospheric degradation in turn leads to a constant deposition of TFA.

Modelling results demonstrate that with constant and ongoing emissions, within a short timeframe (months) the concentration of TFA reaches a steady state, and that the concentration of TFA does not further increase over time. In the case study of the Rhine basin, TFA concentrations below 0.6 µg/L are predicted. The modelled TFA concentrations are well in line with the actual measured TFA concentrations in the Rhine River as reported by UBA considering that the measurements include all sources of TFA, not only those from HFO degradation.

The steady state TFA concentrations are at least 100 times below the German drinking water guidelines (60 µg/L) and are multiple orders of magnitude below the no-effects level of the human health risk assessment (NOAEL = 10 mg TFA/kg bw/day, from a 90-day oral study in rats).

In conclusion, this study invalidates the incorrect assumption made in the REACH PFAS Restriction Proposal that freshwater TFA concentrations would continue to increase until “inevitably” reaching a toxic level.

#### **1.14.PC Unravelling the Complexities of PFAS: From Environment to Human Health and Reproduction**

## Track 2. Ecotoxicology Becomes Stress Ecology: From Populations to Ecosystems and Landscapes

### 2.01 Advancing Risk Assessment of Anthropogenic Stressors to Non-Target-Arthropods and Ecosystem Functions in a Changing World

#### 2.01.T-01 Key Steps and Development for the Revision of the Guidance Document on Non-target Arthropods

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Introduction: Regulation (EC) No 1107/2009 requires a specific risk assessment for non-target arthropods (NTAs). Currently, the NTA risk assessment is performed according to the SANCO Guidance Document (GD) on Terrestrial Ecotoxicology. EFSA PPR Panel (2015) indicates numerous limitations with this GD and risk assessors from Member States frequently highlight their concerns with the inadequacy of current methods and a lack of clarity in the level of protection. Arthropods are fundamental entities of food networks and preserving both their biomass and diversity is pivotal to safeguard ecosystem services delivery. Following a prioritisation exercise that was endorsed by the Standing Committee on Plants, Animals, Food and Feed in January 2023, the revision of the SANCO GD was identified as of high priority. In this presentation, EFSA will summarise the key steps and scientific development needed to undertake the revision of the GD.

Proposed procedure and main challenges: Pesticides regulation states that plant protection products may be approved only if they pose no unacceptable effects on biodiversity and ecosystems. However, the term 'unacceptable' is not further defined in the regulation, and this constitutes a very generic protection goal. EFSA PPR Panel (2010) and EFSA Scientific Committee (2016) proposed a methodology to define specific protection goals (SPGs). Setting SPGs is a crucial step for the GD revision. It requires a dialogue between risk assessors and risk managers, who make the final decision; agreed SPG will then be used as reference for developing risk assessment methodology. Once the SPGs are agreed, the second step will be the development of a protocol which will help making a transparent planning by identifying the problem formulation to be addressed, the methodology to be followed, the resources required and the estimated timelines. Having a pre-defined protocol will allow for different strategies to be followed for addressing the needs (and for adjusting the scope of the GD in the light of the available data while identifying knowledge gaps and needs for further research activities.

For the uptake of the GD in the regulatory risk assessment, the engagement with experts from Member State authorities and stakeholders across the various steps is considered fundamental. Collection of feedback during the revision process will allow to manage expectations and address the feasibility of the GD.

#### 2.01.T-02 Assessing Non-target Terrestrial Arthropods in European Agriculture: A Comprehensive Study on Pest Control Ecosystem Service

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Non-target terrestrial arthropods (NTAs) found on soil or plants play a crucial role in biodiversity and ecosystem services (ES) by naturally controlling crop pests through predation or parasitisation. The taxonomic similarity between NTAs and pests makes them vulnerable to insecticides, prompting the need for evaluating the risk assessment. This study aims to identify and compare the distribution of NTAs providing pest control ES in EU agriculture, analysing variations across different crops, EU geographical zones, and within-crop habitats. The study concentrated on six annual crops (wheat, maize, barley, oilseed rape, potato, beet) and three perennial crops (apple, grape, olives) chosen for their significant contribution to EU production and data availability. Arthropod information for arable crops was sourced from an existing arthropod database [1], while a literature review was conducted for perennial crops by using CAB Abstracts platform. The dataset, focusing on EU-based invertebrates on or above the soil, was refined by excluding pest species, and non-metric multidimensional scaling analysis was used to compare NTAs compositions. Representative NTA families were identified based on criteria such as abundance, dominance, diversity, and distribution. Current pesticide testing strategies, which often focus on standard species like mites or parasitic wasps, face limitations in adequately assessing the diverse biodiversity crucial for pest control. The study emphasizes the extensive taxonomic diversity of NTAs in agroecosystems, revealing patterns in their composition across different crops, EU zones and within crop habitat. A comparison revealed similarity within rather than between major crop types, although this grouping was tighter for perennial crops than for arable crops. These groupings persisted when the data were analysed by EU geographic zones or within-crop habitat. Further examination aimed at identifying key NTA families shows that there are some that are either ubiquitous (e.g. *Carabidae*), occurred sporadically in both crop categories (e.g. *Lycosidae*) or only in one of them (e.g. *Gnaphosidae* and *Pteromalidae*). The findings underscore the necessity for a more precise and ecosystem-service-oriented approach in developing risk assessment guidance for pesticide use in agriculture.

[1] Riedel Jet al. 2016. EU. EFSA Supporting Publications, 13, 956E.

#### 2.01.T-03 Off-Crop Non-Target Terrestrial Organism Drift Exposure and Risk for Plant Protection Products: Processes, Procedures and Practical Proposals

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Developing an effective understanding of the potential for plant protection products to impact non-target terrestrial organisms requires consideration of a range of processes that will influence exposure. This includes better understanding of the role of application techniques, the influence of the target and non-target environment characteristics, and how these effect drift as a key route of entry, as well as ecological/behavioural characteristics of the organisms themselves. Key challenges for these assessments include development of a practical framework that addresses process variability and uncertainty within inherently diverse non-target environments and allows for practical implementation at a meaningful ecologically relevant resolution for a range of organisms. Recently, there has been an initiative from EFSA and Wageningen University and Research to support development of plant protection product exposure assessment scenarios for non-target terrestrial organisms. This project is intended to support guidance development for in-field and off-field exposure assessment associated with spray drift where there are currently considered to be deficiencies. This presentation summarises key differences between conventional two-dimensional deposition/sedimentation spray drift considered in current risk assessments and the more complex challenges associated with interactions with three-dimensional vegetation structures. Various research needs are highlighted: these are required to support development of an effective and meaningful exposure and risk assessment. Appropriate resolution of assessment and strategies for addressing variability are deemed key considerations. Primary challenges include mechanistic representation of spray drift, characterisation of vegetation capture of drift and the implications of spatial variation of exposure in field margins (vertically, with depth and laterally along field interfaces). A truly effective and meaningful risk assessment framework also needs to take into account non-target organism distribution, development and life stage vulnerability, and ecological characteristics such as mobility, routes and duration of exposure. This presentation will summarise research efforts underway within CropLife Europe of relevance to these challenges that may offer practical contributions towards development of a coherent, practical and meaningful basis for tiered risk assessment.

#### **2.01.T-04 Integrating Multiple Exposure Routes for Assessing Pesticide Impact on Terrestrial Non-Target Arthropods**

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To ensure the protection of terrestrial non-targeted arthropods (NTAs), conducting comprehensive environmental risk assessments (ERAs) for pesticides is crucial. Currently, Tier 1 risk assessment frameworks often rely on standardized test species such as *Aphidius rhopalosiphi* and *Typhlodromus pyri*, evaluated on glass plates focusing on freshly dried residues post-application. However, NTAs encounter pesticides through diverse routes in natural settings—contact with dried and fresh residues on plant surfaces, direct overspray, and oral exposure through contaminated food. Moreover, each test employs distinct units (e.g., chemical per individual, per surface area, and concentration in food items), which hampers a holistic understanding of pesticide risks in the actual environment.

Recent advancements have demonstrated the potential for establishing an integration framework to encompass different exposure routes for bees. This framework used a toxicokinetic-toxicodynamic model (TKTD), i.e., the BeeGUTS framework. This model discerns between kinetic effects (uptake and elimination) and dynamic effects (intrinsic sensitivity), unveiling organism sensitivity irrespective of exposure routes. As a result, such an approach allows for unified interpretation of results from diverse exposure routes. In other words: knowing the actual outcome of a chronic oral test, will allow predictions of the outcome of a future acute contact test.

Our study aims to implement a TKTD approach, akin to BeeGUTS, to explore three distinct exposure routes (acute contact, acute indirect, and chronic oral) involving *Gryllus bimaculatus* toward imidacloprid. Our experimental design involved exposing the organisms to direct chemical exposure, surface exposure, or oral ingestion in three separate experiments. Through the utilization of TKTD models, we interpret data, calibrate, and validate the model for untested exposure routes, leading to the development of a comprehensive model capable of integrating various exposure pathways. Ultimately, our study endeavours to advance the integrated framework for assessing pesticides on NTAs, underscoring the significance and feasibility of incorporating multiple exposure routes when evaluating the pesticide impact on these organisms.

#### **2.01.T-05 Development of Ecotoxicological Testing Methods on Herbivorous Larvae of *Locusta migratoria* and *Spodoptera littoralis* under Laboratory Conditions**

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Non-target arthropods (NTA) can be affected by Plant Protection Products (PPP) via different exposure routes. Besides 'overspray' and 'contact' there is also the oral exposure route via residues on food. In the current environmental risk assessment of Plant Protection Products (PPP) to non-target arthropods, oral exposure via food ingestion by herbivorous species is not considered and therefore, no standard laboratory test protocols for oral exposure of arthropods other than bees are available. In the EFSA Scientific Opinion of 2015 on NTA's, *Lepidoptera* and *Orthoptera* are two of the suggested taxa to cover this herbivorous oral route of exposure. There are few studies with both taxa, which are representative for NTAs, indicating that a comparison of contact versus both oral and contact is not always possible.



In May 2022 CropLife Europe NTA expert sub-group initiated the development of an ecotoxicological testing method to assess side effects of plant protection products on herbivorous *Lepidoptera* and *Orthoptera* via oral exposure route. For both species a main test was conducted and evaluated in two different GLP laboratories. The main tests comprised the exposure phase of 48 hours (acute mortality) as well as the development period (sublethal effects) and reproduction assessment.

The objective of this presentation is to present the results of these ecotoxicological test methods developed for oral exposure with *Spodoptera littoralis* and *Locusta migratoria* and evaluate criteria to assess suitability and reliability of both test species for an oral exposure test system. The criteria are control mortality, sensitivity, food consumption / oral uptake, handling, test duration, challenges, sex determination and feasibility of reproduction assessments. Acute and chronic endpoint were assessed and statistically evaluated for both species. The ERx values will be presented for *Spodoptera* and *Locusta* in comparison.

## **2.01.P Advancing Risk Assessment of Anthropogenic Stressors to Non-Target-Arthropods and Ecosystem Functions in a Changing World**

### **2.01.P-Tu064 The Effects of Landscape Structure and Pesticides on the Populations of Non-Target Arthropods in Germany**

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The recent decline in the diversity and abundance of insects has been linked to intensified agriculture and simplified landscape structures as well as the intensive use of pesticides. Pesticides, in particular, have been shown to affect the populations of Non-Target Arthropods (NTA) both in-field and off-field, with their impacts influenced by several factors, including landscape structure. To disentangle the links between landscape structure, pesticide use and their effects on NTA populations, land use/cover and agricultural data from two German federal states, Brandenburg and Lower Saxony, were examined. The selected study areas showed significant variation in the composition and configuration of landscape elements (e.g. field size, natural habitats) in the two states. ALMASS (Animal, Landscape and Man Simulation System) modelling framework was utilised to simulate the impact of pesticide on NTA populations (represented by *Bembidion lampros*) and the effectiveness of selected mitigation measures: grassy field boundaries (FB) and unsprayed field margins (UM). Under the baseline scenario (no changes to landscape structure and no pesticides applied), significant variation in *B. lampros* populations was observed across study areas and explained by landscape characteristics. The addition of FB led to an average increase of 9.8% in beetle populations across all study areas. Under the pesticide scenario, beetle populations decreased significantly in all study areas (average 14%), and the effects varied depending on landscape structure. The impacts were higher in homogeneous landscapes with low initial beetle populations, high arable land coverage and low source habitat coverage. The beetles were also affected in exclusive off-field areas where pesticides were not applied or drifted. The simulations showed that both, FB and UM, reduced the effects of pesticides. However, FB were a more effective measure than UM. Our results indicate that pesticide use affects *B. lampros* populations also through source-sink dynamics, resulting in significant off-field effects that persist even with mitigation measures in place. The findings show that the existing risk assessment of NTA has significant shortcomings and that the existing measures for mitigating risks are insufficient. It is recommended that the management of agroecosystem landscapes focuses on establishing, preserving, and safeguarding habitats for insects, particularly in homogeneous agricultural landscapes.

### **2.01.P-Tu065 An approach to include more realism in the assessment of human impacts on species abundance and diversity**

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Current risk assessment schemes focus on a very defined aspects of the risk posed by chemicals applied by farmers and other human activities, e.g. the risk is considered for one product at a time and the assessment focuses on a very defined calculation of exposure, which does not reflect the full range on conditions in agriculture. Furthermore, also changes of agricultural practice, habitat loss, climate change related impacts and other factors become more and more important. Hence on one side, the relatively narrow view of the risk assessment scheme may not indicate a risk for those substance approved for the market, and on the other side, studies from scientist show an alarmingly rapid loss of biodiversity. And it often remains unclear, which factors drive that loss mostly. In the present study we present a landscape scale approach to evaluate which factors play a main role for the presence of species. Aerial images of the same landscapes from Germany from 2003/2004 and 2018 were used to evaluate changes of landscape structure over time. After automated digitisation of these aerial images using our software ALDiS, arthropod abundance was simulated and the effects related to changes of landscape structure were compared to those resulting from application of hypothetical pesticides. We discuss the use of this relatively simply screening approach to analyse potential reasons of changes in species abundance and diversity.

### **2.01.P-Tu066 Consideration of Sublethal Effects of Insecticidal Substances in Plant Protection Products or Genetically Modified Plants on Non-target Invertebrates**

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Systemic insecticides and insecticidal substances produced by genetically modified crops (GMP) share that non-target

invertebrates can be exposed via the diet, either by feeding directly on crop material or by feeding on herbivores which have fed on the crop. Especially dead or less mobile pest organisms can be an easily accessible food resource for invertebrate omnivores and predators. Since insecticidal substances can be produced over the full life-time of such a GMP and systemic insecticides are designed to protect the crop over prolonged time periods, non-target organisms can be chronically exposed, partly in low doses. Hence, this type of exposure may lead to sublethal rather than lethal effects. We evaluated for which invertebrate taxa which sublethal endpoints are addressed in the first tier effect assessment of plant protection products (PPP) in the EU and compared this with current practice in the effect assessment of GMP with insecticidal properties. For PPP, exposure via diet is routinely tested only for the honeybee. In addition to bees and surrogate soil organisms, the lower tier effect assessment for PPP considers only 'beneficial' non-target arthropods, a predatory mite and a parasitoid wasp. Testing of a non-target herbivore is actually not required for PPP and the focus of tests is often on short-term exposure via overspray and contact. For GMP environmental risk assessment, no fixed data requirements are defined. Selection of test species and test protocols are often derived from risk assessment but modified to address exposure via diet. Survival is almost always assessed but often also growth and/or development are monitored. Analysis of reproduction is much rarer, but this may be partly explained by the mode of action of most GMP assessed so far. In , we identified a lack of consideration of sublethal effects on non-target invertebrates in case of potential chronic exposure. Thus, we think that the time is ripe for a cooperative action of scientists from both, the PPP and the GMP community, to collect experiences gained in the past with different test protocols and to initiate the preparation of refined or new test guidelines, especially for non-target arthropods.

### **2.01.P-Tu067 The Interspecies Sensitivity of Non-Target Terrestrial Arthropods (NTAs) for Pesticides: Species Vulnerability and Exposure Route**

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The first Tier of the pesticide environmental risk assessment for non-target terrestrial arthropods (NTAs) has a data requirement on effect studies with two standard test species involved in pest control, the predatory mite *Typhlodromus pyri*, and the parasitoid wasp *Aphidius rhopalosiphi*, using contact exposure to dried spray residues on glass plates. Several other NTA species are commonly used if higher Tier assessment is required, applying standardized laboratory experiments using contact exposure-effect studies using dried residues on natural substrates, such as leaves or soil. The EFSA (2015) Scientific Opinion on NTA risk assessment addressed various concerns on the risk assessment framework, e.g., that these generic species and safety margins may not sufficiently cover interspecies sensitivity of vulnerable species that are key drivers for various Ecosystem Services (ES). It was also noted that for bee risk assessment, exposure routes are based on dietary exposure and topical contact, which would also relate to many other (pollinating) NTAs. The ongoing AENEAS project has identified key driver NTA families for various agricultural crops, for ES pest control and pollination. Our literature search indicated that studies on species sensitivity distributions (SSDs) for NTAs are extremely scarce, while toxicity endpoints are abundantly reported in published literature and active substance dossiers for many NTA species. One major issue is that NTA exposure routes and durations vary strongly, hampering strict SSD comparisons. Using CAB Abstracts, Hertfordshire PPDB, and EPA-Ecotox Knowledgebase, surface contact lethal rates (LR50) and topical lethal doses (LD50) were retrieved, in order to compare generic test species endpoints to those for NTA from selected families. Defined LR50 endpoints for the two standard NTA in PPDB showed that for 34% of 131 chemicals, sensitivity differed between 10-18000x. Other NTA species listed in PPDB for 46 pesticides showed endpoints with lower sensitivity than the two generic species for 17%. From the EPA-database, surface contact SSDs were derived for 18 pesticides (>5 species), for comparison to generic NTA species. From a topical LD50 review database, SSDs were derived for 32 pesticides (>5 species), for comparison to honeybee endpoints. Most data-rich pesticides, however, are no longer allowed for use in, e.g., Europe, but do allow for evaluating sensitivity ranking and comparison to different exposure routes.

### **2.01.P-Tu068 The Impacts of Nitrogen Dioxide Exposure on Insect Fitness**

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Tropospheric pollutants generated by anthropogenic activities pose a significant threat to public and environmental health. Previous research has focused on their link to human disease; however, the ecological effects of air pollution remain poorly understood. Insects are a highly diverse group that play a critical role in ecosystem functioning, but are showing patterns of decline, particularly in industrialised countries. The links between pollutant exposure and insect fitness have not yet been thoroughly investigated. This work aims to characterise insect responses to air pollution, focusing on the direct effects on individual fitness. Using fumigation chambers, we will expose aphids, moths, and lacewings to ecologically relevant concentrations of nitrogen dioxide (NO<sub>2</sub>), and observe growth, development, fecundity, and survivorship. We hypothesise that NO<sub>2</sub> will have a deleterious impact on the growth, development, and fecundity of moths and lacewings, with mortality rate increasing with pollutant concentration. We predict this will be driven by oxidative stress and cellular damage, and will perform glutathione-S-transferase (GST) assays to confirm the mechanism of toxicity. In contrast, we also hypothesise that aphids could benefit from higher concentrations of NO<sub>2</sub>, based on previous research demonstrating population increases under poor air quality. This study will provide vital and novel understanding of the consequences of air pollution on biodiversity and ecosystems and grants scope to further investigate the cascading effects on trophic levels. We hope that by enhancing the

existing knowledge base, this research will contribute valuable ecological insights to inform future air pollution policy making and legislation.

### **2.01.P-Tu069 *Episyrphus balteatus* Adult Acute Overspray Toxicity Protocol**

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The use of plant protection products (PPPs) is crucial for modern agricultural practices, but when performed in an extensive manner, it can negatively impact important non-target arthropods (NTAs), essential for a functional and healthy ecosystem. Pollinators, foliage and ground dwelling predators, parasitoids and several other ecosystem service providers are exposed to pesticides applied in crops. The lack of studies on exposure of NTAs to PPPs via overspray contact makes it difficult to draw a clear picture of what might be the possible consequences of PPP use. Also, the data retrieved from standard ecotoxicological tests might not be very useful for the development of population models necessary to understand long-term effects. With this study we aim to elaborate a protocol that can be applied to different NTA species and help us to collect data to assess effects of PPPs derived from an overspray application and also to collect extra data that could feed TK/TD models. The developed protocol was tested with *Episyrphus balteatus* to assess its feasibility and its applicability to different NTAs. Pre-exposures practical aspects like the time needed for adults to hatch (to guarantee sufficient number to perform a valid ecotoxicological test), the number of individuals per replicate, the diet and type of feeders, the time that they need to be exposed to the CO<sub>2</sub> to be anesthetized, and the overall time that is needed to complete all the procedures, were tested. After being anesthetized and before exposure, individuals were placed in a spraying cage (10 per cage) weighted and a photograph was taken to know precisely the amount of substance that will come into contact with each specimen. Using a Potter tower, organisms were sprayed allowing a more even distribution of the solution across the pretended area, namely on top of our test organisms, and the cages were weighted again and the individuals were transported to clean arenas. During the exposure period several cages were sampled over different time periods (instead on only one time) to collect data for TK/TD models. Survival was assessed and live organisms were used for residue analysis to assess internal body burdens, linking external exposure dose with internal concentrations. This test allowed us to optimize the protocol for further test species of NTAs from different functional groups.

### **2.01.P-Tu070 Evaluation of the exposure to triazole fungicides in arthropods**

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The use of pesticides in agriculture has emerged as a major cause of biodiversity loss in agroecosystems. Triazole fungicides are among the most widely used pesticide groups nowadays. The mechanism of action of these substances consists of impeding the formation of the fungal wall by inhibiting the synthesis of ergosterol, one of the wall's main components. In arthropods, the alteration of the sterol biosynthesis pathway by triazoles may be interfering with the production of ecdysteroids, a type of hormones involved in moulting and reproduction processes. This study evaluated the impact of triazoles on arthropods through two experimental exposures simulating field scenarios: (1) ingestion of treated cereal seeds and (2) ingestion of treated leaves. Two arthropod models, mealworms (*Tenebrio molitor*) and silkworms (*Bombyx mori*), were employed.

For exposure to treated seed, mealworms were exposed to ground barley seeds treated at 100, 50 and 25 % of the Raxil® Plus (tebuconazole and prothioconazole) application rate mixed with untreated barley seed. For exposure to foliar treatments, silkworms were exposed to treated mulberry leaves previously dipped for ten seconds in different concentrations of Folicur® (tebuconazole): 2.5, 1, 0.5, 0.1 ml/L. Food consumption, weight at each stage, development time and reproductive parameters were evaluated in both experiments.

On exposure to treated seeds, mealworms showed aversion to treated seeds, especially in the intermediate treatments, and experienced a decrease in weight. No significant differences in development time were observed and reproductive parameters could not be evaluated. As for foliar exposure, exposed silkworms showed lower leaf consumption, lower weight and delayed pupation compared to controls. In addition, an increased proportion of individuals failed to form cocoons in the higher concentration treatments. No significant differences in egg fertility were found.

These results indicate that experimental exposure to triazoles, at levels similar to field conditions, affects the growth of mealworm larvae and the development of silkworms. It is suggested that steroid-disruption effects related to the use of these pesticides could impact arthropod populations in agricultural environments, emphasizing the need to better understand the implications of triazoles on biodiversity and ecosystem functioning.

### **2.01.P-Tu071 A test design to assess the oral exposure of the herbivorous lepidopteran larvae *Plutella xylostella* to pesticides under controlled laboratory conditions – Pros and Cons and further challenges**

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In the EU, the risk assessment for non-target arthropods (NTAs) follows the framework developed by ecotoxicologist in the ESCORT workshops. In 2015 EFSA published a Scientific Opinion (SO) to address the request of the EU commission to review the existing framework. Different ways of exposure to pesticides are one of the aspects that were addressed in the SO

asking for an additional test species. In particular, the oral exposure through ingestion of contaminated food was identified as a challenge since the current available endpoints from a tier 1 test design cannot be related to oral toxicity and cannot be used in a risk assessment for this exposure mode. Therefore, the working group of the PPR panel recommended to develop assessment methodologies for focal species such as herbivorous NTAs. Hence, there is the need to develop a reproduceable methodology to enable us to evaluate the oral toxicity of PPP to lepidopteran larvae, for example.

Additional to other initiatives followed by the CLE NTA working group, we started working with one common lepidopteran species to gain first experiences which will guide the further development of methodologies. The project's purpose is to develop a test method to be used under Good Laboratory Practice (GLP) in the laboratory. Criteria for the selection of test species were defined and *Plutella xylostella* (Plutellidae, Lepidoptera) was identified as a suitable test organism. Different exposure matrices and diets were tested during first trials. Key factors related to validity criteria like control mortality, Larval feeding behavior, larval development time, adult hatching rate and egg production in the control were evaluated.

The advantages and disadvantages of *P. xylostella* as an organism for which laboratory-based breeding techniques are well established, also for efficacy trials, will be highlighted.

We will provide first recommendations for the development of a testing protocol and present results to illustrate the technical feasibility and expected challenges in developing assessment methodologies for oral exposure using an herbivorous arthropod like *P. xylostella*.

### **2.01.P-Tu072 Urban gardens as important small-scale habitats for insect species - in vitro and in vivo methods indicate chemical pollution**

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Insects are experiencing a drastic biodiversity and biomass decline, while providing important ecosystem functions. Therefore, it is of great concern to further uncover causes for species loss, with the goal of identifying mechanisms to dampen species loss. The interdisciplinary project "SLInBio" (<https://www.insektenvielfalt-frankfurt.org/>) is investigating how the perception and valorization of insects can be increased in Frankfurt am Main and what contribution cities can make to the conservation of insect diversity. Garden owners supposedly hold the potential to influence small-scale habitat quality: in urban areas, private gardens display a relevant amount of the total green area distributed among the city, hence gardens pose important habitats for organisms. But individual practices and preferences in aesthetics and the use of pesticides and fertilizers, chemical run-off from building materials, water management or animal keeping can strongly influence the habitat quality and thus insect biodiversity. As an ecotoxicological approach to this subject, sediment and water samples were collected from ponds from 12 gardens, distributed among the city of Frankfurt am Main. The samples were tested in vivo with the non-biting midge *Chironomus riparius* in a sediment-water system. Emergence success varied strongly between the samples and was compared to in vitro assays performed on water and sediment extracts. A significant correlation was found between Microtox data from water samples and emergence success of midges: water samples with higher toxicity showed lower emergence success in their corresponding sediment-water system and vice versa. Other parameters like pH, temperature, ammonium, organic fraction or particle sizes could not explain the different emergence rates in the samples. Furthermore, a non-target was performed on the water samples to identify chemicals that could possibly explain this observation. The results show that toxic substances can affect populations of merolimnic insect species, even in small water bodies maintained by gardeners.

This study aims to help improve habitat quality of small-scale habitats for insect species by hopefully uncovering feasible changes gardeners can make on their private property to support local insect diversity.

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### **2.01.P-Tu073 Ants as Model Organism to Evaluate the Ecological Risk of active plant protection substances? Lethal and Sublethal Effects of the Systemic Neonicotinoid Imidacloprid in Three Ant Species**

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To minimize potential risks to non-target organisms, including ecologically beneficial ants, pesticides have to undergo toxicity risk assessments. However, ants (Hymenoptera: Formicidae) are not included in any type of risk assessment for non-target organisms. Ants are important components of most terrestrial ecosystems and provide crucial ecological services such as pollination, soil improvement and nutrition cycling. They have developed many specialized traits to thrive in various ecological niches and have evolved complex feeding ecologies, e.g. fungus cultivation, seed harvesting, omnivory, or pure predation. These distinct feeding ecologies expose ants to different types of pesticides, especially in agricultural areas where pesticides are applied to manage pest organisms that can compromise crop yield.

In this study, we developed a testing strategy with different levels of complexity and present a method to evaluate the impact of pesticides on three ant species. We used the systemic neonicotinoid imidacloprid to assess both lethal and sublethal effects on three ant species which can be maintained in the laboratory. To do so we orally exposed subsets of 50 minor worker ants to seven different Imidacloprid concentrations dissolved in 10%-honey water. The results revealed that Imidacloprid has an LC<sub>50</sub> of 8.42 mg/L (4.72 -16.18 mg/L 95% confidence interval) for the model species *Camponotus maculatus* over a 10-day period. Similar LC<sub>50</sub> concentrations were observed for *Lasius niger* (11.03 mg/L (6.30 – 21.93 mg/L 95% confidence interval)) and *Crematogaster sp.* (3.97 mg/L (3.27 – 4.84 mg/L 95% confidence interval)). Furthermore, analysed internal Imidacloprid concentrations in deceased and survived individuals using UPLC-MS/MS after QuEChERS extraction and calculated LD<sub>50</sub> values to approximate the lethal body burden and the tolerance range of surviving specimens.

In an identical follow up experiment (extended exposure period of 21 days), we also included larvae (20 per 50 worker subset). While there was lethal effect of imidacloprid on larvae at concentrations which did not affect the workers, we found a disruption of the metamorphosis process during early pupation. In particular, 38% of the surviving larvae failed to create a functional pupal cocoon. Considering our results and the proven feasibility of using ants as study organisms, we see a good potential for including ants in future risk assessment studies.

## **2.01.P-Tu074 Unveiling the Ecotoxicological Impacts of Realistic and Worst-Case Scenarios of Plant Protection Product Mixtures on the survival of *Chironomus riparius***

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Plant protection products (PPPs) are intensively employed in agriculture to ensure crop yields, with 466 pesticide-active ingredients approved within the European Union (EU). This excessive overuse leads to a substantial part ending up in water systems, compromising their quality. Despite the well-documented adverse effects of PPPs, a predominant focus on individual compounds overlooks the real-world application scenarios, where PPPs are applied as mixtures resulting in the input of approximately 300 billion kilograms of PPPs mixtures into waters. This stands out the need for a comprehensive understanding of the collective impact of PPP mixtures on the environment. This study, conducted within the framework of the H2020 SPRINT project, aimed to assess the impact of realistic and predicted PPP mixtures, on the survival of the aquatic insect *Chironomus riparius*. For this purpose, 11 case study sites (CSSs) were selected, within each, the concentration of PPPs was measured in aquatic systems near agricultural fields. A mixture of 5 PPPs was delineated for each CSS, according to their frequency of occurrence and the risk quotient (RQ). Following the OECD test guideline 235, larvae of *C. riparius* were exposed to each condition: measured environmental concentration (MEC), predicted environmental concentration (PEC) 3\*PEC and 5\*PEC, for each mixture. Of the total of 11 mixtures, only one (CSS2) provoked significant differences in mortality for all conditions and for PEC, 3PEC and 5PEC in the case of CSS1 and CSS6. Moreover, significant differences were detected after 3PEC and 5PEC for CSS3, CSS4, CSS7, CSS9, and CSS11. By contrast, lower toxicity, only by 5PEC, was observed for the CSS8 and CSS10. The environmentally relevant conditions MEC and PEC, altered mortality on CSS1 and CSS6, spite of having concentrations lower to the LC<sub>50</sub> values for each PPP, reinforce the heightened risk of PPP mixtures in aquatic systems. Furthermore, in the worst-case scenario (5PEC), a 100% mortality rate was observed across all CSSs. These findings emphasize the urgency of identifying the co-occurrence of distinct PPPs in real scenarios and studying their effects on non-target aquatic organisms. Additionally, it is imperative to incorporate more realistic approaches, considering PPP mixture with diverse modes of action, given the diverse effects associated with the specific mixture employed. **Funding:** EU Horizon 2020 (grant 862568); UNED (REGAGE21e00018296847: A.B.M.G).

## **2.02.A Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment**

### **2.02.A.T-01 Chronic multigenerational radionuclide exposure in *Lemna minor*: from epigenetics to population**

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It is the purpose of this project to study the long-term effects (over multiple generations) at different levels of biological complexity (from molecular to population level) in a duckweed species (*Lemna minor*) exposed to environmentally relevant concentrations of different genotoxic radionuclides linked to possible nuclear accidental scenarios (137Cs, 90Sr). The organism *Lemna minor* was chosen for this project as it is a fast vegetative-growing macrophyte, the only freshwater plant for regulatory toxicity testing for chemicals, and a model organism within ecotoxicology.

The Chernobyl nuclear accident in 1986 has given rise to a region characterized by the presence of multiple radionuclides, with 90Sr and 137Cs still persisting nowadays in the Chernobyl Exclusion Zone (CEZ). Therefore, we investigated the long-term effects of these genotoxic radionuclides on the molecular physiology of the aquatic plant *Lemna minor*.

In our study, we employed a 6-week multigenerational setup and worked with both high activity concentrations (kBq/l) and ecologically relevant ones (Bq/l). Data from qPCR analysis of chromatin remodeling (SRT1), methyltransferases (DRM3, MET1, ROS1), glutathione biosynthesis (GR, GSH1, GSH2.1, GSH2.2), and telomere length regulation (TERT) genes show time-specific gene expression trends differing upon exposure to different radionuclides.

Results indicate that morphological trait analysis (frond area analysis), photosynthetic activity (DUAL pam measurements), pigment and glutathione level measurements, and enzyme activity (GPOD) analyses support the hypothesis of a putative hormetic ionizing radiation response specific to either 90Sr or 137Cs contamination. *Lemna minor* may differentiate its abiotic stress responses towards either 90Sr or 137Cs by funneling energy respectively into epigenetic regulation or redox balance maintenance.

In conclusion, by employing molecular biology (gene expression analysis) and non-conventional ecotoxicological endpoint testing, this project contributes to expanding current scientific knowledge on *Lemna minor*'s radiation stress biology with the final aim of environmental risk assessment amelioration.

#### **2.02.A.T-02 Amendments Improve Performance of Perennial Native Plants for Revegetation of Acidic Mine Tailings Over Time**

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In the US there are over 20,000 abandoned hardrock mines that may present risks to human health or the environment from long-term exposure to harmful substances. One site is the Formosa mine Superfund site located in Oregon, US. The site lacks plant cover due to phytotoxic tailings with high acidity, possible metal toxicity, insufficient nutrients, and poor soil structure which make it difficult to establish vegetation. We evaluated the effects of amending the tailings on tailing chemistry, and survival and growth of local native herbaceous species: *Anaphalis margaritacea* (ANMA), *Carex inops* (CAIN), *Chamerion Angustifolium* (CHAN), *Elymus glaucus* (ELGL), and *Potentilla gracili* (POGR). The treatments were (1) withholding any amendments, (2) amending with lime, biosolids, and biochar, (3) amending with lime, biosolids, and K fertilizer, and (1), (2) or (3) plus inoculation with locally-sourced microbes (LSM). Amendments increased pH and reduced available heavy metals in the tailings, and improved plant survival and growth. The grass, ELGL, had greater survival and growth than the other species with the amendments. Plant biomass during the fifth year of growth was greater for ELGL with the amendments with the biochar treatment than the amendments with the K treatment. In contrast, the other species had greater biomass with the amendments with the K treatment. There were no LSM effects to date, though evaluation is continuing. Thus, soil amendments can improve soil chemistry and enhance native herbaceous survival and growth on mine tailings.

#### **2.02.A.T-03 Measuring the effect of time variable concentrations on green microalgae: a laboratory comparison study**

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Due to their ubiquitous presence in the environment, green micro-algae play a central role in the risk assessment of Plant Protection Products (PPP). OECD guideline 201 provides a clear protocol for how the response of these organisms to constant exposure scenarios can be routinely tested in a laboratory setting. However, due to the rapid growth patterns that quickly deplete the nutrients in the test medium, it has proven far more challenging to test longer time variable exposure patterns. We present the preliminary results of a laboratory comparison test that evaluates the robustness and reproducibility of two distinct methodologies for generating data for time-variable exposures tested on green microalgae. One method is a semi-static approach that relies on a series of filtration and resuspension steps allowing the testing of time-variable exposures in a test regime that approximates the OECD 201 guideline. The other method is a flowthrough chemostat system that, once steady state has been reached, can be used to test long and complex exposure patterns. Both methodologies can be used to address important risk assessment questions, and can also serve to validate TKTD models, thus expanding the possibilities of refined risk assessment.

#### **2.02.A.T-04 Reproducibility, Reliability and Regulatory Relevance of Terrestrial Plant Visual Injury (PVI) assessments**

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The authorization of plant protection products (PPP) in the EU requires an assessment of potential impacts on non-target terrestrial plants (NTTP) taking into account quantitatively assessed parameters like biomass, survival or emergence. Recently, considering an ER<sub>50</sub> derived from visually assessed plant injuries (PVI) in the risk assessment has been requested in addition. The PVI Working Group (WG) within the SETAC Plants Interest Group is aiming to support the determination of regulatory endpoints for PVI, and foster harmonization in PVI assessments, ultimately strengthening the environmental impact

assessments for PPPs and the protection of NTTP. In a first step, current challenges were determined (e.g., by conducting surveys in laboratories that are assessing PVI regularly). The utilization of a non-harmonized scoring system for the quantification of PVI (e.g., chlorosis, necrosis, wilting etc.) relies on subjective human observations, making results less reliable and objective. In addition, the survey results revealed that there is a lack of guidance concerning how PVI assessments are to be conducted with regards to both scoring methods as well as statistical evaluation, yet both are needed to reliably determine an objective ER<sub>50</sub> for PVI. Various PVI scoring methods (e.g., 0-4; 0-5; 0-10 rating groups) exist that encompass both percentage ranges or categorical descriptions, leading to varying interpretations. The method of converting scores into percentage values affects the 50% effect level definition, as does the statistical model (e.g., regression model or Cochran-Armitage test) for ER<sub>50</sub> determination. Due to the subjectivity of the assessment, the variation in the scores assigned to an individual plant by different assessors can be substantially. Given the diversity of PVI assessment methods, it is often challenging to objectively determine whether the PVI ER<sub>50</sub> is lower than other endpoints and accordingly should be used to conduct the risk assessment. To address these challenges, the WG is actively pursuing a standardization of PVI data interpretation, acknowledging the various assessment methods. This endeavor includes evaluating data from different methods and comparing outcomes through different statistical tests. In the future, the WG aims to develop new assessment methodologies, standardized PVI scoring systems, and appropriate statistical analyses including ring testing to evaluate the viability of a new approach.

### **2.02.A.T-05 Simulating ecosystem services of natural plant communities of importance for indirect effects on Endangered Species: the IBC-Grass model as a case study**

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In the USA, Endangered Species Assessments (ESA) are of critical importance to ensure pesticides are used in such a way that ensures protection of non-listed and endangered species. Besides direct effects of pesticides on endangered species, effects on biotic elements on which endangered species rely on for e.g., food and shelter are critically important for a successful ESA. Among those biotic elements, the plant community that provides both food and shelter to the rest of the ecosystem is probably the most evident biotic element that, if affected by pesticide use, can result in detrimental indirect effects on endangered animals (such as terrestrial arthropods, mammals, birds, etc.)<sup>1</sup>. In this work we explore a framework for model plant communities in field margins and some of their ecosystem services through mechanistic plant community modelling. For this, we use an Agent-Based plant community model (the IBC-Grass model)<sup>2</sup> that simulates a grassland plant community through the definition of plant functional types. As the modelling is based on plant functional types rather than specific plant species, it can be considered to represent plant attributes that are generic to any plant species if the species belong to a similar functional type (e.g., small rosette vs tall plants, etc.). The model can represent various plant functional types and their interaction through competition for resources above and below ground, and the effect of agronomic practices (such as annual plowing, mowing, etc.) in the plant interactions. A module to represent effects of pesticides based on standard plant toxicity studies is also incorporated in the model. In the presentation we will explore a framework to use this model in the context of indirect effects on endangered species. We will explore how endpoints for effects on some of the ecosystem services linked to indirect effects on endangered species (such as shelter and food provision) could be derived and used in the context of ESA assessment of pesticides.

### **2.02.B Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment**

#### **2.02.B.T-01 Ecotoxicological Test Protocol for the Assessment of Reproductive Endpoints in Non-Target Terrestrial Plants under Greenhouse Conditions**

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Ecotoxicological testing to assess the effects of plant protection products on non-target terrestrial plants (NTTPs) under greenhouse conditions is conducted according to standard guidelines (e.g. OECD 208 and OECD 227). EFSA's Scientific Opinion of 2014 on the risk assessment of plant protection products (PPP) for non-target terrestrial plants raised awareness of a potential need to assess effects on plant reproduction. Literature reviews and experimental studies evaluated the feasibility and relevance of plant reproductive endpoints. In summary, it can be concluded that (a) assessment of reproductive endpoints in selected plant species is in principle feasible, (b) there is no clear trend that reproductive endpoints are more sensitive than vegetative ones, and (c) cases may occur where reproductive endpoints appear to be more sensitive depending on, e.g., mode of action of a plant protection product and/or test species. For cases where effects on reproductive endpoints need to be assessed for regulatory purposes, a standard test protocol is required. A working group within the SETAC Plant Interest Group collected and analyzed relevant and available data and experiences to provide an ecotoxicological test protocol for the assessment of reproductive endpoints in Non-Target Terrestrial Plants under greenhouse conditions. This presentation will provide an overview of the protocol and will highlight the challenges and special considerations such as test duration, plant species and endpoint selection as well as statistical evaluation.

#### **2.02.B.T-02 Microalgal Responses to Organic Contaminants and Influence on Contaminants Fate in Aquatic Ecosystems**

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Effects of organic contaminants (OCs) on microalgal physiology were extensively studied in the last years. However, a knowledge gap exists regarding the role of microalgae on OCs fate. Such information is required to better assess contaminants persistence and their trophic transfer, to identify biomarkers of contaminants exposure and to improve microalgae-based water remediation strategies. Genome sequencing of model microalgal species revealed the presence of genes encoding for enzymes (e.g. Cytochrome p450) involved in OC biotransformation in other organisms such as bacteria, higher plants, animals. However, knowledge about biotransformation pathways in microalgae lag far behind that of other microorganisms.

In our study the marine diatom *Phaeodactylum tricornutum* was exposed to three OCs, two pharmaceuticals (Diclofenac, Ethynilestradiol) and the herbicide Diuron. OC effects on cellular traits and physiology were studied together with OC removal from the exposure medium (HPLC-UV) and OCs biotransformation (HPLC-MS).

The lowest observed effect concentration (LOEC) varied considerably between pharmaceuticals (0.3-1 mg/L) and the pesticide (3 ug/L) and different modes of toxic action were described for the three contaminants. Diuron inhibited cell growth and photosynthetic activity, Diclofenac effected membrane integrity but not through generation of oxidative stress. Finally, Ethynilestradiol induced oxidative stress and altered cell morphology with lipid droplets formation. *P. tricornutum* cultures were shown to be able to influence pharmaceuticals fate. Significant removal from the exposure medium was observed for Ethynilestradiol (up to 60% for the tested concentrations after 72h exposure) while transformation products were identified for Diclofenac.

Our results confirm that phytoplankton can influence the fate of contaminants in the water column. However, this process depends on the OC and its modes of toxic action. Ongoing analyses on transcriptomic (RNA-seq) data will shed light on the activated pathways possibly involved to respond to OCs exposure through biotransformation and compartmentalization processes.

#### **2.02.B.T-03 Functionalisation and size dependence effect of upconverting lanthanide doped nanoparticles, NaYF<sub>4</sub>:Yb,Er@NaYF<sub>4</sub> on wheat seedlings' growth, germination, cell death and membrane permeability**

**Anna Ekner-Grzyb, Department of Cell Biology, Adam Mickiewicz University, Poznan, Poland**

In recent years, nanotechnology has become widely utilized. Nanoparticles (NPs) are applied in various fields, including medical applications, plant fertilizers and cosmetics. One of the rapidly developing materials are inorganic nanocrystals doped with lanthanide ions, which possess distinctive spectroscopic properties, including luminescence capability, resistance to photobleaching and degradation, extended luminescence lifetimes and narrow absorption and emission bands. Moreover, the NPs exhibit efficient anti-Stokes emission (known as up-conversion), converting NIR or IR radiation into visible emission. These properties minimise autofluorescence from biological tissues, and cells and enhance signal detection sensitivity, which is one of the primary challenges in bioimaging. Additionally, eliminates harmful UV radiation exposure to biological materials.

However, these NPs may enter the environment and influence biota. Consequently, there is a need to investigate their influence on living organisms. Therefore, our study focuses on analysing the toxic effects of two core@shell lanthanide-doped upconverting nanoparticles (NaYF<sub>4</sub>:Yb<sup>3+</sup>,Er<sup>3+</sup>@NaYF<sub>4</sub>), with different surface modifications (poly(ethylene glycol)-neridronate, PEG-Ner and poly(acrylic acid), PAA) and sizes (26 nm and 52 nm). We examined the impact of the NPs on germination percentage, germination rate, growth and cell membrane damages and cell death of wheat seedlings (*Triticum aestivum* L.), the species being one of the most extensively cultivated crops.

In summary, the study demonstrated that non-modified NPs, regardless of size, the most effected the wheat seedlings. PAA-modified NPs exhibited toxicity, particularly at smaller sizes, while PEG-Ner-modified UCNPs had no toxic impact. Consequently, PEG-Ner was identified as the safest surface compound among the investigated UCNPs, potentially mitigating the adverse effects of nanoparticles on plants. Moreover, whole plants were imaged using an up-conversion laser scanner, successfully detecting all tested NPs inside.

#### **2.02.B.T-04 Ecotoxicological Effects of Copper and Pendimethalin at Three Different Temperatures on *Raphidocelis subcapitata*- the Impacts of Climate Changes**

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In the last decades, climatic changes have been a great concern among the scientific and political communities. Nowadays, climatic changes effects have been reported associated to extreme climatic events like the raise of temperature, floods and droughts with impacts in several activities (e.g. agriculture) and in the ecosystems. The exponential increase in world population and growth in food consumption are placing unprecedented demands on agriculture and natural resources. Hence,



more research is needed, not only for a more diverse array of pesticides, but also for commercial formulations, applied in the field.

Fitoplanktons (ex: *Raphidocelis subcapitata*) are a key biological group that produce essential nutrients vital for the survival of higher trophic levels, being the main source of food. Copper belongs to the group of transitional essential metals, of vital importance for every organism at low concentrations, becoming toxic at high amounts, being considered the third most toxic metal after cadmium and mercury. Pendimethalin is a worldwide used herbicide part of the dinitroaniline family. It is used to control annual grass and broadleaf weed in various crops and in residential lawns.

Hence, this work aims to determine the combined effect on the growth of *Raphidocelis subcapitata* of a range of temperatures (15°C, 20°C and 25°C) on the toxicity of two pesticides.

Both compound were tested using OECD 201 growth inhibition test, with some modifications. Total number of cell in each replica was obtained through spectrophotometric absorbance at 440 nm. As expect in the three tested temperatures pendimethalin proved to be more toxic than copper to *Raphidocelis subcapitata*. In the case of copper Kruskal-Wallis's test was used to determine the significance of each parameter. In the case of pendimethalin a Two-way ANOVA followed by Tukey's test was used to determine the significance of each parameter. In both cases statistical tests pointing to both temperature and concentration and indicating the combination of temperature and concentration are significant.

These studies are of the highest importance in our actual context, since they allow us to predict the potential toxic effects of these two compounds in freshwater primary producers. Further research should focus the mixture of these two compounds since this can provide a more realistic approach of the interactions that occur in the environment.

#### **2.02.B.T-05 Developing a Reference Tier with Quantitative Aquatic Macrophyte Field Data**

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It has been identified that there is a strong need to establish ecological baseline responses as a means to create workable tools for the risk assessment of aquatic macrophytes. This project aimed at collating quantitative field data to characterize the baseline biotic and abiotic parameters in the edge-of-field water bodies. The project collected information from freshwater edge-of-field aquatic ecosystems to identify which macrophyte species grow in edge-of-field water bodies (ponds, streams and ditches) and which species can be considered as characteristic species; which abiotic conditions prevail in edge-of-field ponds, streams and ditches and which can be considered as key conditions and are also relevant for macrophyte models; and which functional traits do these characteristic macrophytes species represent. We followed a stepwise methodology to meet the project aims. First we explored available databases at the European level, openly published datasets and datasets available in The Netherlands and surrounding countries of the central zone to select a group of 30 macrophyte species characteristic for edge-of-field ponds, streams and ditches. Secondly we explored databases and published literature to extract the key conditions under which these macrophytes grow under field conditions, how wide they are distributed over Europe, how often they are used in Species Sensitivity Distributions and microcosm and mesocosm studies and which growth forms and functional traits they represent. The data show that most of the macrophyte species characteristic for ponds, streams and ditches have a wide distribution in Europe. The characteristic species show a large overlap with those tested in potted plant studies in mesocosms. The selected aquatic macrophytes grow in circum-neutral surface water (average pH 7.4). The water is buffered (average alkalinity 2.6), medium to high in nutrient content and medium in salinity and chlorinity. This project results in a database with 30 aquatic macrophytes, their scientific and common names, growth forms, some functional traits, presence in the three selected categories of water types as well as genus, family, order, use in SSDs or micro- and mesocosm studies, and surface water, pore water and sediment quality at the stands of these aquatic macrophytes. The collated database can serve to support a reference tier for aquatic macrophyte risk assessment and to support macrophyte models.

#### **2.02.P Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment**

##### **2.02.P-Th083 Uptake and Toxicity of Per- and Polyfluoroalkyl Substances (PFAS) at Environmentally Relevant Water Concentrations by *Azolla filiculoides***

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Environmental contamination of aquatic systems by per- and poly-fluoroalkyl substances (PFAS) have generated significant health concerns. Remediation of contaminated sites such as fire-fighting emergency training grounds that used Aqueous Film Forming Foams are of a high priority and phytoremediation may help play a part in removing PFAS from associated contaminated waters. In this study we investigate the potential of the water fern, *Azolla filiculoides*, to uptake seven common PFAS during a 12-day exposure to environmentally relevant concentrations delivered as equimolar mixtures: low ( $\sum\text{PFAS} = 0.0123 \pm 1.89 \mu\text{mol L}^{-1}$ ), medium ( $\sum\text{PFAS} = 0.123 \pm 2.88 \mu\text{mol L}^{-1}$ ) and high ( $\sum\text{PFAS} = 1.39 \mu\text{mol L}^{-1}$ ) treatments, equivalent to ~5, 50 and 500  $\mu\text{g L}^{-1}$  total PFAS respectively. Possible phytotoxic effects of PFAS were measured at 3-day intervals using chlorophyll *a* content, photosystem II efficiency ( $F_v/F_m$ ), performance index and growth rate. PFAS concentrations in plant tissue and water were measured every three days using ultra-high performance liquid chromatography - tandem mass

spectrometry. PFAS exposures did not lead to any detectable phytotoxic effects that may negate the use of *A. filiculoides* in phytoremediation. All seven PFAS were detected in plant tissue with most uptake occurring during the first six days of exposure. At three days post treatment, a maximum bioconcentration factor was recorded for PFHxS of 9.9 and minimum of 2.8 for PFBA. Consequently, the application of *Azolla* spp for phytoremediation of PFAS from aquatic environments is limited but does show potential as part of an integrated water treatment system.

## **2.02.P-Th084 Ecotoxicological Assessment of Four Veterinary Antibiotics (Enrofloxacin, Doxycycline, Tylosin, and Lincomycin) on Terrestrial and Aquatic Indicators**

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Antibiotics play a crucial role in treating bacterial infections in both humans and animals. However, their extensive usage has led to their presence and accumulation in the environment, raising concerns about potential adverse effects on aquatic and terrestrial organisms. This study aims to assess the ecotoxicological impact of four prominent antibiotics—enrofloxacin, lincomycin, doxycycline, and tylosin—chosen for evaluation due to their status as among the top ten most consumed antibiotics in Europe. Understanding their environmental impact is particularly vital given their significant role in the livestock industry.

Three organisms were selected as indicators for this study: *Daphnia magna*, *Eisenia foetida*, and *Allium cepa*. These organisms are widely employed in assessing antibiotic ecotoxicology. *D. magna*, a freshwater crustacean, is known for its sensitivity to various environmental contaminants. *E. foetida*, a soil-dwelling worm, is commonly used to evaluate chemical toxicity in soil organisms. *A. cepa*, a plant species, is sensitive to diverse environmental stressors.

In terms of toxicity to *D. magna*, the antibiotics ranked from most to least toxic are enrofloxacin (272.93 ppm), doxycycline (287.44 ppm), tylosin (523.20 ppm), and lincomycin (2564.12 ppm). For *A. cepa*, doxycycline (18.61 ppm) exhibited the highest toxicity, followed by tylosin (21.06 ppm), lincomycin (45.56 ppm), and enrofloxacin (78.88 ppm). All compounds showed an EC<sub>50</sub> exceeding 1000 ppm in the *E.foetida* test.

Furthermore, the toxicity of the four antibiotics studied closely aligns with that of other antibiotics detected in the environment, such as chlortetracycline, oxytetracycline, and florfenicol. Previous research has shown similar toxicity levels against *D. magna* (200-300 µg/ml), *A. cepa* (94 µg/ml), and *E. foetida* with EC<sub>50</sub> values exceeding 1000 µg/ml.

These findings suggest that acute toxicity effects of these four antibiotics are unlikely to manifest in these indicator organisms, as the concentrations required to induce toxicity are considerably higher than those observed in the environment (ng/l to µg/l). Additional ecotoxicity tests spanning multiple trophic levels, including community-level assessments and extended-duration studies, are imperative to comprehensively evaluate potential chronic effects.

## **2.02.P-Th085 Seasonal Dynamics of Phytoplankton Biodiversity, Abundance and Biomass in the Freshwater Lentic Mesocom System Located in Southern Poland**

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Static mesocosm systems are used to assess the risk posed by introduction of chemicals into water and soil matrix. The mesocosm system is carried out under field conditions and mimics natural ecosystems, which allows for a comprehensive assessment of the effects of a chemical on selected, closely related aquatic ecosystem parameters. The analysis of phytoplankton populations in mesocosm studies can be crucial when assessing the effects of herbicides.

Due to the field nature of the study, the aim of the presented project was to analyse the seasonal variability of phytoplankton population during one production season in relation to selected physico-chemical parameters. The test was performed in stainless steel tanks, settled in the ground (about 2400 L each tank), situated in Southern Poland. The tanks were supplied with an appropriate amount of sediment and water and inoculated by adding sediment and water from natural reservoirs. Sampling was carried out from April 2022 to September 2022. The project included qualitative and quantitative (biomass) phytoplankton analysis, physico-chemical analysis of water i.e.: temperature, pH, oxygen concentration, conductivity, nitrites (NO<sub>2</sub><sup>-</sup>), nitrates (NO<sub>3</sub><sup>-</sup>), phosphate (PO<sub>4</sub>), ammonium (NH<sub>4</sub><sup>+</sup>) and Total Organic Carbon (TOC).

During observations, the number of taxa verified to at least genus level ranged from 53 to 96 depending on the tank. The total abundance of taxa indicated an increase in Euglenozoa and Cynaprocaryota genera from June onwards and the occurrence of Cryptophyta only in April, May and September. In addition, there was a peak in the taxa number of Chlorophyta and Bacillariophyta in the first half of June. Myzozoa were present throughout entire vegetation period but the taxa were mainly limited to *Certium hirundinella*.

Shifted biomass levels, observed during temperature increases, were recorded from the end of July and emerged in relation to the mixed-type blooming by Cyanoprocaryota (*Dolichospermum crassum*) and Myzozoa (*C. hirundinella*). The increase in *D.*

*crassum* biomass was associated with the increase in turbidity and a decrease in the number of Chlorophyta taxa. Moreover, blooming elevated oxygen, TOC and phosphate concentrations.

These findings improve the interpretation of the risk experiments results, and furthermore they could implicate mesocosm designing processes.

#### **2.02.P-Th086 Further Evaluation of Macrophytes for Species Sensitivity Distribution (SSD) Tests**

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The OECD guideline 239 assessing the rooted aquatic plant *Myriophyllum spicatum* and the OECD guideline 221 assessing *Lemna* sp. free-floating species are followed to evaluate toxicity in macrophytes. By now it became obvious that the focus on one submerged rooted and one free floating species might under, or overestimate effects based on the mode of action of test items. Therefore, further tests with additional species were setup to refine risk assessment. This leads to the general question: what other species show good growth under lab condition and can be tested based on the available guidelines? Following this question, we performed tests with non-standard species, including monocotyledons and dicotyledons, emergent, submersed, and free-floating species, to refine risk assessment. We present data of non-standard macrophyte test species which based on doubling time and coefficient of variation fulfilled validity criteria according to OECD 239 or OECD 221.

#### **2.02.P-Th087 First Approach to Compare Sensitivity of Macroalgae and Symbiosis-Living Microalgae to Standard Test Species**

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Macrophytes and microalgae are highly sensitive to pollutants. This highlights the importance of including photosynthetic active organisms in risk assessment. However, there are other photosynthetic organisms that have been less studied, namely, macroalgae and symbiosis-living microalgae, such as microalgae living in corals.

In this work, we present results on their sensitivity to 3,5-dichlorophenol which is used as reference standard for standard test species. Based on the mode of action of the pollutants, we will present evidence that macroalgae species and microalgae living in symbiosis with corals have a high sensitivity. These data could be useful to further evaluate the importance of considering additional photosynthetic organisms for risk assessment.

#### **2.02.P-Th088 Study on the Effects of Particulate Matter on Plants According to Exposure Routes**

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The research on the impact of particulate matter on the environment and human health continues to expand, with a growing interest, particularly in its effects on plant ecosystems. Particulate matter can settle from the atmosphere onto plant surfaces or accumulate in plants through the soil, potentially causing adverse effects on plants. This study aimed to evaluate the impact of particulate matter on plants, taking into consideration various exposure pathways. Furthermore, to assess the impact of particulate matter on plants based on leaf morphology, we conducted a comparison between cabbage, characterized by fine outgrowths or appendages (trichome) on its leaves, and lettuce, which lacks such features. To assess the impact of particulate matter on plants, we evaluated various factors, including plant growth, photosynthetic efficiency, ROS effects, and leaf stomatal size. This study provides vital information for comprehending the effects of particulate matter pollution on plant ecosystems and for devising measures for environmental conservation and plant protection.

#### **2.02.P-Th089 Using Flow Through Mesocosms to Investigate How Herbicide Exposure Impacts Macrophyte Communities and Long Term Recovery**

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Small water bodies (SWBs) are commonly located on or near agricultural land, meaning that macrophyte communities can be exposed to herbicides that enter SWBs via spray drift, surface runoff and/or subsurface drainage. Macrophytes provide a number of important ecosystem services, such as positively influencing biogeochemical cycles and functioning as ecosystem engineers.

Currently, most research focuses on the immediate in-season impacts on aquatic macrophytes from exposure to single herbicides, but does not consider longer term recovery, the influence of mixtures or the impact of herbicides on plant communities.

This is the first long-term study to use state-of-the-art flow-through mesocosms situated at FERA Ltd in York, UK. Here, we bring together researchers from different institutions and regulatory bodies to answer the following questions:

- What are the community level effects after exposure to a herbicide dose that exerts a clear effect on individual macrophyte species?
- What is the nature and timescale of recovery for individual species following an impact?
- How does exposure to a mixture of herbicides change the nature of community impacts and/or the timescale of recovery?

Ten mesocosms are being studied over an 18-month period. Eight emergent and one submerged macrophyte species are included within the mesocosms to represent a realistic SWB community. Six mesocosms are dosed with the sulfonylurea herbicide metsulfuron-methyl in successive spring seasons, and three of these mesocosms are also dosed with a mixture of autumn-applied herbicides in the autumn following the first metsulfuron-methyl treatment. Macrophyte recovery is being measured using fortnightly recordings of: chlorophyll fluorescence, number of leaves and flowers, and length of the longest stem. Community composition is being measured using aerial photographs to calculate percent cover with data being analysed using principal response curves.

The results of this study will be used to assess the necessity and feasibility of incorporating recovery potential and community-level impacts into herbicide risk assessment.

**2.02.P-Th090 Temperature related sensitivity of the green alga *Ankistrodesmus falcatus* exposed to Diflufenican**  
*Melanie Grolms-Aal<sup>1</sup>, Eric Bruns<sup>2</sup>, Johannes Witt<sup>2</sup>, Tido Strauss<sup>1</sup> and Jutta Hager<sup>2</sup>, (1)gaia Research Institute, Germany, (2)Bayer AG - Crop Science Division, Germany*

Ecotoxicological tests with primary producers, such as planktonic algae, contribute to a better understanding of the environmental impact of pesticides (herbicides) and to an informed risk assessment. In general, during the last years, the ERA is increasingly focused on non-standard organisms. Also, more realistic environmental conditions are applied as refinement option to address the effects in the environment more accurately.

This study was based on OECD 201 (2011) and aimed to determine the influence of the herbicide Diflufenican on exponentially growing populations of the unicellular green alga *Ankistrodesmus falcatus* under different temperature and light conditions.

Four experiments were performed at 8°C, 11°C, 17°C and 23°C. Light regimes were adapted to these temperatures to avoid light inhibition at low temperatures and to obtain natural, and therefore physiologically meaningful, light and temperature combinations for the laboratory experiments. This was achieved by combining field data on global radiation and water temperature. All tests were conducted under static exposure over 96 h and increasing Diflufenican concentrations at lower temperatures. Growth was quantified daily by microscopic cell counts and fluorescence measurements.

Diflufenican exposure was stable over time at all temperatures. As expected, growth generally decreased with lowering temperature for both endpoints. When considering the effects of exposure, a more differentiated pattern emerged. Lower sensitivity and delayed growth inhibition to Diflufenican with decreasing temperature were observed in the cell count results. Furthermore, fluorescence levels increased during the first few days of treatment before showing almost the same effects as cell counts.

Rapid detection of growth by biomass is the advantage of fluorescence measurement. Cell counting provides information about cell division. Therefore, both endpoints, cell count and fluorescence measurements were relevant. Exposing algae to lower temperatures requires extending the duration of the test to detect growth inhibition.

This study demonstrates how uncertainties in ERA can be reduced by better understanding the mechanisms leading to the effects of herbicides. Integrating seasonal light and temperature conditions in laboratory experiments allow a more reliable extrapolation to the field.

**2.02.P-Th091 Residue assessment of toxic metals in seaweeds for livestock feeds**

*Jin Seong Kim, Kang Sang Woo, Oh Kyeong Yeol and Kim Jin Hyo, Agricultural Chemistry, Gyeongsang National University, Korea, Republic of (South)*

Seaweed is known to contain 10-20 times higher minerals than terrestrial plants, and is interested a useful alternatives for feed sources. Furthermore, it has been reported that a few seaweeds can reduce methane emissions from livestock up to 30%, and the value for feed are increased. However, seaweed can be a potential source of heavy metal, thus, the contents of toxic metals (As, Cd, Cr, and Pb) in the commercially available six species of seaweeds were analyzed by ICP-OES. From the experiments, Cd, Cr, and Pb were not detected in *Ecklonia cava*, *Chrysymenia wrightii*, *Undaria pinnatifida*, *Porphyra tenera*, *Ulva lactuca* while *Codium fragile* was detected Cd, Cr and Pb as total ions at 0.1 mg kg<sup>-1</sup> 0.74 mg kg<sup>-1</sup>, and 2.5 mg kg<sup>-1</sup>, respectively. In addition, As detected in ranging on 6.6 - 87.1 mg kg<sup>-1</sup> in the all six species of seaweeds.

**2.02.P-Th092 Survey of bromoform contents in seaweeds on the Korean coast by GC-μECD**

*Kang Sang Woo, Jin Seong Kim, Oh Kyeong Yeol and Kim Jin Hyo, Agricultural Chemistry, Gyeongsang National University,*

Korea, Republic of (South)

Methane exhibits a global warming potential 28 - 34 times higher than CO<sub>2</sub>, and ruminant is known to a major methane emission source. Reduction of methane emission are currently issued in the livestock industry, and bromoform is known to a potential inhibitor for methanogenesis in rumen. A red algae, *Asparagopsis taxiformis*, was known for the potential methane production inhibition, and bromoform as a trihalomethane (THMs) was found in the seaweed. However, the potential toxicity of bromoform as a feed additive were concerned. In this study, bromoform contents including (chloroform, chlorodibromoform, dichlorobromoform and iodoform) were investigated in six commonly used seaweed species (*Undaria pinnatifida*, *Codium fragile*, *Porphyra tenera*, *Ecklonia cava*, *Ulva lactuca*, and *Chrysymenia wrightii*). Freeze-dried seaweed samples were extracted using methyl tert-butyl ether and the extracts were analyzed by GC- $\mu$ ECD. The results showed that bromoform was not detected in any of the six seaweeds. However, chloroform was detected to 1.5 – 37.0 mg kg<sub>dw</sub><sup>-1</sup> in the six seaweeds. THM contents were not considerably high in the seaweeds.

#### **2.02.P-Th093 Investigation of the residue dissipation and pre-harvest residue limit for sethoxydim and fluzifop-p-butyl as an herbicide in root minor crops.**

**Kim Jin Hyo, Jin Seong Kim, Oh Kyeong Yeol and Kang Sang Woo, Agricultural Chemistry, Gyeongsang National University, Korea, Republic of (South)**

Fluzifop-p-butyl and sethoxydim were known as selective herbicides and these were not registered for minor crop application. In South Korea, 17.5% fluzifop-p-butyl as emulsifiable concentrate (EC) and 20% sethoxydim as EC were registered and these were use for the investigation of magnitude of crop residue for critical GAP. In this study, the two pesticides were applied to twice to surface soil in minor root crops, and the residues were investigated in the leaf and root. In the root, both of sethoxydim and fluzifop-p-butyl were not detected trace amount on below 0.01 mg/kg when applied before 20-days of harvest, while trace amounts of the herbicides were detected below 0.01 mg/kg in the leaves. Thus, the MRL establishment were not suggested when the root minor crops cultivation practice were considered. The theoretical maximum daily intakes of the two herbicides in South Korea were calculated to be 4.5-10.8% of ADIs.

#### **2.02.P-Th094 Sewage sludge long-term fertilization: effects on heavy metal accumulation in soil and willows (*Salix viminalis* L.)**

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The use of renewable energy resources is one of the high priority areas in the EU policy in order to decrease greenhouse gas emissions and to increase energy independence. Short rotation energy forestry, i.e. the production of fast growing tree species, is one of the alternatives for energy production. Wastewater treatment generates huge amounts of sewage sludge (Ss) and its production in the world is projected to increase in the future. Sustainable sewage sludge management and disposal is a growing problem worldwide. The main risks of using SS are related with heavy metals (HM), persistent organic chemicals and high content of nutrients. This study aimed to evaluate long-term fertilization (15 years) with SS impact on soil quality and heavy metals accumulation in soil and multi-purpose energy plant willow (*Salix viminalis* L.). Willow plantation was fertilized twice in the period of cultivation: 20 t/ha of SS in 2011 and 100 t/ha in 2016. Plants were harvested in 2011 and in 2020. The following parameters were evaluated of soil samples and willow cuttings made after fifteen years of cultivation (2005-2020): accumulation of heavy metals in soil, shoots and leaves of willows, bioconcentration and translocation factors.

#### **2.02.P-Th095 Phytotoxicity Effects of Tetracyclines and Sulfonamides mixture in presence of copper on *Lemna x mediterranea* plants**

**Chiara De Carolis<sup>1</sup>, Marco Fabriani<sup>2</sup>, Giovanna Frugis<sup>2</sup>, Fabrizio Pietrini<sup>3</sup>, Valentina Iori<sup>2</sup>, Maria Letizia Costantini<sup>4</sup> and Maria Adelaide Iannelli<sup>2</sup>, (1)Sapienza University of Rome, Italy, (2)Institute of Agricultural Biology and Biotechnology, National Research Council (IBBA-CNR), Italy, (3)Italian National Research Council (IRET-CNR), Italy, (4)Dipartimento of Environmental Biology, Sapienza Università di Roma**

Aquatic environments have become one of the main destinations of antibiotics, with significant negative effects on ecosystems. Pharmaceuticals limit the habitable living environment for aquatic organisms and antibiotics, considered as emergent contaminants, contribute to cause severe water bodies pollution. Duckweed (*Lemna x mediterranea* 9425a), a common aquatic plant found worldwide, was grown in a solution polluted with sulfamethoxazole (SMX-sulfonamide group) and chlortetracycline (CTC-tetracycline group) with low copper concentrations (Cu) usually present in moderately polluted environments. Following OECD guideline protocols<sup>1</sup>, *L. mediterranea* 9425a plants were tested as monocultures with different concentrations of antibiotics, alone, mixed and in presence of copper sulphate to simulate environmental conditions. The adverse effects on *Lemna* sp. are concentration- and time-dependent for growth inhibition, photosynthetic capacity decrease and induction of oxidative stress response. The inhibitory effects induced by SMX, CTC and Cu on duckweed plants have been evaluated by measuring the number and size of fronds, wet/dry fronds biomass, relative growth rate (RGR), pigment content and chlorophyll fluorescence analysis. Concentration-response relationships were defined to estimate toxic endpoints of Cu, EC<sub>50</sub>: 7.27 mg/L; SMX, EC<sub>50</sub>: 173.00 mg/L and CTC, EC<sub>50</sub>: 6.9988 mg/L since their presence, in aquatic environments, may have significant implications for tested organisms and their ecosystems.

<sup>1</sup>OECD. *Lemna* sp. growth inhibition test. Guideline 221. Organization for Economic Co-operation and Development: Paris, France 2006.

## **2.02.P-Th096 Challenges in calculating the mean measured concentrations for Algal Growth Inhibition Test According to OECD Test Guideline 201 - And what about the toxicity?**

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Green algae, blue-green algae and diatoms (tested following the OECD TG 201) are at least partly important for the risk assessment of plant protection products, human and veterinary pharmaceuticals and industrial chemicals under REACH. Especially chemicals as basic elements for the synthesis of more complex products show a broad variety of characteristics from well water soluble, stable and non-toxic to hardly water soluble, unstable, volatile and very toxic for water organisms. The group of chemicals with one or more of the latter characteristics is therefore a challenge for toxicity tests with aquatic organisms. The OECD GD 23 for difficult substances provides some hints how “standard testing” with such test items should be conducted, but due to numberless combinations of characteristics of these substances, some innovation is required to find the best test design for individual chemicals. Substances that degrade rapidly or are volatile in particular pose a challenge for testing but also for interpreting the results. When testing algae, a semi-static test is difficult to carry out and involves a lot of effort. Therefore, the test is usually carried out statically. In the case of volatile substances, a closed system can be used. However, in experiments with rapidly degradable substances, the recovery can be very low after a test period of 72 hours. For these substances, it is advisable to measure the degradation or loss of the substance every 24 hours, to obtain data at test start, after 24, 48 and 72 hours. In case of very low values measured 24 h intervals (< limit of quantification), the mathematically formula chosen to calculate the mean measured concentrations is very decisive since it will give them more or less weight. Therefore, the challenge is to correctly calculate the mean value from these different values, which is important for risk assessment. To our knowledge, there are still no binding specifications from authorities as to how this should be calculated. Each version of the OECD GD 23 proposes a different type of calculation, but there is no GD summarizing of all possibilities. It should also not be forgotten that the toxicity and thus the growth of algae can be affected by the degradation or offgassing of the substance. This has also not yet been considered in the calculation. Various options for calculating of geometric mean measured concentrations and the determination of EC<sub>50</sub> values for growth and biomass are explained and discussed.

## **2.02.P-Th097 Paper-disc Soil Method to Predict Ex-situ Soil Quality Evaluation**

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Soil algae are potential soil test species due to being representative terrestrial organisms in terms of ecologically important producer, wide distribution, and ease of cultivation. The paper-disc soil method is a soil algal bioassay for assessing soil water transferred from the contaminated soils. In this study, we performed the paper-disc soil method for predicting *ex-situ* soil quality evaluation. Test media were contaminated soils collected from the smelter sites and remediated soils. The test species were *Chlorococcum infusionum* and *Chlamydomonas reinhardtii* and algal photosynthetic fluorescence was measured to indicate biomass. We verified different chlorophyll *a* effects depending on soil properties. This result suggests the application of the paper-disc soil method to *ex-situ* soil quality evaluation. *Acknowledgement-This work was supported by Korea Environment Industry & Technology Institute (KEITI) funded by Korea Ministry of Environment (MOE) 2022002450002 (RS-2022-KE002074).*

## **2.02.P-Th098 Photosynthetic Effects of Polyvinyl Chloride Microplastics in Paints**

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Polyvinyl chloride (PVC) is an additive for a paint to prevent the attachment of illegal flyers. Paint particles can be naturally or artificially fragmented via deterioration or removal from the surface coating materials. In the end, PVC can be contaminated the areas adjacent or distant to the source after weathering, aerial dispersion, or deposition of paint particles. In this study, we assessed the photosynthetic effects of PVC microplastics as an additive in paint on a plant mung bean. Photosynthetic effects on *Vigna radiata* were conducted by following OECD test guidelines No. 208 and assessed in seven exposure system: 0, 0.01, 0.1, and 1% paint only (paint), and 0.01, 0.1, and 1% paint mixed with PVC microplastics (P + PVC). Significant differences between paint and P + PVC treatments were showed in Fv/Fm, ABS/RC, TRo/RC, ETo/RC, and Dio/RC. This result suggests that PVC microplastics in paints enhance disruption of photosynthetic mechanism in plants. *Acknowledgement- This work was supported by Korea Environment Industry & Technology Institute (KEITI) funded by Korea Ministry of Environment (MOE) 2022002450002 (RS-2022-KE002074).*

## **2.02.P-Th099 Toxic Effects of Three Detergents on Cladophora Sp. (Chlorophyta)**

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Detergents are products that are discharged in large quantities into aquatic systems, causing harmful effects to species. Various toxicological studies have been carried out with microalgae exposed to surfactants and detergents, however, studies with filamentous algae are scarce, for this reason, the objective of this work was to evaluate the effects of three types of detergents: a dishwasher (DW), a laundry detergent (DL) and a multipurpose detergent (DM); in growth, production of pigments, production of macromolecules (proteins, carbohydrates and lipids) and degree of lipoperoxidation in the filamentous algae *Cladophora* sp. to evaluate their sensitivity to these compounds. Static tests of seven days duration were carried out, where the algae were exposed to 5 concentrations of the surfactant sodium dodecyl sulfate (0, 0.01, 0.1, 1, 5, 10, 50 and 100 mg/l) and to 5 concentrations of the three commercial detergents (0, 1, 15, 30, 45 and 60 mg/l). At the end of the exposure period, growth (wet weight), production of pigments and macromolecules and the degree of Lipoperoxidation were evaluated. The EC<sub>50</sub>

values varied from 0.7 to 18.06 mg/L, the most toxic detergent was the multipurpose detergent. A decrease in the concentration of total chlorophyll was observed in algae exposed to detergents of between 2% and 68%. Exposure to SDS and commercial detergents cause changes in the production of macromolecules and an increase in the production of TBARS of 10%. 300% compared to the control group. The degree of toxicity of the detergents ranged from highly toxic to harmful.

### **2.02.P-Th100 Reassessing Exposure Design: Exploring Overspray in Toxicity Tests for Herbicides with Unexpectedly Low Toxicity to Macrophytes**

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Herbicides are commonly classified based on their mode of action, which has implications for their toxicity to aquatic macrophytes. While some herbicides, including contact herbicides, exhibit localized toxicity, there are instances where contact herbicides unexpectedly show low toxicity to emergent and floating macrophytes compared to systemic herbicides. This raises concerns about the protectiveness of current exposure designs in toxicity tests. Standard tests for macrophytes typically involve dissolving the herbicide in the test media to ensure even exposure. However, contact herbicides primarily act through direct contact with non-submerged parts of the plants. As a result, the use of overspray exposure, involving spraying or drift, may provide a more appropriate method for assessing the toxicity of contact herbicides specifically to emergent and floating aquatic macrophytes.

Previous research has indicated increased sensitivity differences in overspray or spray drift exposure scenarios for aquatic macrophytes.

This study investigates the potential influence of overspray exposure on the estimation of toxicity for contact herbicides. To accomplish this, we subjected *Lemna minor* to the contact herbicide Bentazone using two distinct exposure scenarios: overspray and exposure via the growth medium. In accordance with the OECD 221 Lemna Growth Inhibition Test, we conducted a dose-response experiment to evaluate differences in biomass and frond area. We reached approx. 90% of the nominal concentrations for the exposure via the medium. Exposure via spray resulted in an approx. 4.5x higher concentration than via the medium, e.g., for the highest dose. Nevertheless, effects on *Lemna* were only observed when the herbicide was dissolved in the medium. We will discuss potential explanations for these results, e.g. related with the uptake of the test item by the lower or upper part of the *Lemna* fronds.

### **2.02.P-Th101 Ecotoxicity evaluation of several composts through *Lactuca sativa*, *Daphnia magna* and *Lemna minor***

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To match the European Green Deal by 2050, the global system must urgently move to contaminant free and zero residues. The agricultural system, through the Farm to Fork strategy, must contribute towards a sustainable food production and to circular economy, particularly shifting from conventional to organic agriculture and limiting mineral fertilization.

Composting the increasing organic residues has arisen as a promising technological option, the resulting bio-products as organic fertilizers available to a huge potential market. Correspondingly, composts are aimed not only to fulfil the standard quality test requirements of their basic chemical composition; they are also to guarantee minimizing their environmental impact. Analyses and routine assays for compost quality are however frequently insufficient to secure absence of effects on the environment.

To assess for the agricultural use suitability of representative composts produced from a diversity of raw materials by composting plants in the North-East area of Spain, a lettuce Petri dish seed germination and early plantlet growth test with two crude compost concentrations, and a standard acute toxicity test of compost leachates on *Daphnia magna* as biosensor to determine the concentration which induced half-maximum effects (CE<sub>50</sub>) were carried out. For most compost samples, germination rate was unaffected, but some of them decreased plantlet development (shoot and radicle height and weight). The *D. magna* neonates exhibited different sensitivities to increasing concentrations of the compost leachates depending on the nature of the composts, even though their EC<sub>50</sub> was high and well over that of the compost concentration intended for their use. Conversely, only one of the composts exhibited a parallel effect both on *D. magna* and on lettuce germination and early plantlet growth. Further bioassays with *L. minor*, are still ongoing. The proposed approach of testing compost impacts on plants and on other ecotoxicity test model organisms is needed to ensure the feasibility of compost use to safely producing agricultural goods without compromising soil fertility. Research towards determining the minimal environmental impact of agricultural compost practices is required to contribute to maximize production in a circular economy context.

### **2.02.P-Th102 Risk Assessment based on Visual Effect Endpoints for Terrestrial Plant (NTTP)**

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The Non-target terrestrial plant (NTTP) risk assessment of chemicals uses ecotoxicological endpoints based on effects on plants (phytotoxicity). Phytotoxicity can be indicated as change in visual appearance of the plants (such as chlorosis, necrosis, wilting, leaf and stem deformation), weight or length. As neither of these parameters alone covers the effects of the other in

either assessment cases, authorities ask to determine ER<sub>50</sub> values for visual effects, emergence, biomass, and shoot length to conduct NTTP risk assessment for plant protection products (PPP).

However, some assessors feel challenged by the use of “visual phytotoxicity” in the risk assessment. We want to respond to two important points. One is that the visual effect value provides an overall picture of the phytotoxic effects by covering the sum of all symptoms appearing in parallel in the plants affected. However, if these effects are treated separately, the overall phytotoxic effect on the plants is not captured, e.g. when chlorosis precedes necrosis in the plant tissue. As herbicides cause different symptoms in the plants which might have a population-relevant effect, we propose to preferentially use the endpoint “visual phytotoxicity” as a sum of effects.

Another issue is that the information to conclude on the effect value depends on the quality of the documentation provided in the studies used to evaluate the PPP risk to NTTPs. However, it is common practice that if no ER<sub>50</sub>-value could be derived from the data, then a minimum effect value (ER<sub>50</sub> > x) will be used in the risk assessment. Although the guideline recommends to present the effects in percentages (0-100%), the studies often provide an alternative scoring system. To deal with that, besides derivation of a minimum ER<sub>50</sub> (> x) based on hypothesis testing for binomial data, often the ER<sub>50</sub> can be derived from the different scores (typically 5, 9 or 10 scores or levels of phytotoxicity). Therefore, firstly each scoring is set to the midpoint of its range. Then effect endpoints (ER<sub>50</sub>) are calculated for these binomial distributed data using common software tools to derive ER<sub>50</sub> values from a dose/effect curve (e. g. R-package drc, implemented in OpenRTox).

We want to share these thoughts on the use of the endpoint “visual phytotoxicity” in PPP risk assessment to support the design of such tests for PPP risk assessment.

### **2.02.P-Th103 Use of *Egeria dense* as a Pb adsorbent in wastewater treatment**

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Water pollution is a global environmental problem; human activities, through industrial and agricultural processes, have caused the degradation of water quality, which generates problems related to the supply of drinking water and wastewater treatment (Lavado-Meza et al., 2020). Currently, there is great concern worldwide, due to the considerable increase in the contamination rates of industrial effluents by heavy metals such as Cr, Ni, Cd, Pb and Hg (Tejada-Tovar & Garcés-Jaraba, 2015). Adsorption is an efficient technique for purifying water, removing low levels of contaminants, and is often advantageous in terms of simplicity and cost-effectiveness. In recent years, the use of low-cost materials obtained from different biomasses from plants, fruits, algae and agro-industrial waste has been investigated to replace the use of conventional methods in the removal of contaminants, such as heavy metals. Said removal is achieved due to the interaction between metal ions with the functional groups present on the surface of the biomaterial. *Egeria dense* or elodea as it is also known, is an aquatic plant that is used in aquariums, to oxygenate ponds and aquariums. On the other hand, other properties have been found such as adsorption and its great metal removal capacity (Martinez-Vasquez et al. 2018). The objective of this project is to study the adsorption of biochar obtained from *Egeria dense* as an alternative process for the removal of contaminants such as Pb. 0.1 g of biochar was used in 25 mL solutions with concentrations from 0 to 300 mg/L of Pb (II). They were brought into contact by stirring for 120 minutes and the retention time of 30 minutes was determined to obtain 72.31% removal and an adsorption capacity of 179.88 mg/L of Pb (II). The results obtained demonstrate that biochar produced from elodea can be useful as an adsorbent for the treatment of water contaminated with Pb (II).

### **2.02.P-Th104 Duckweed (*Lemna minor*) Response to Triclosan and its Recovery after Exposure under Elevated Temperature**

*Diana Miškelytė and Jurate Zaltauskaite, Vytautas Magnus University, Lithuania*

Antimicrobials are important for preserving the quality of life and public health. Due to the rising amounts of antimicrobial agents in the environment and the lack of knowledge on their ecotoxicity, there is growing concern regarding their effects on the terrestrial ecosystem. Triclosan (TCS), an antibacterial chemical used extensively in pharmaceutical and personal care products, is also an often-recognized emerging organic pollutant in the environment. Physiological or morphological endpoints are used in most published studies assessing TCS toxicity to aquatic plants. Additional detailed research is needed to understand the underlying toxicity mechanisms of TCS-induced effects at the biochemical plant level. With potentially far-reaching consequences for life on Earth, climate change is an increasingly urgent issue. There is a growing awareness of the importance of anticipating the interactions between natural and chemical stressors, and the way they affect organisms and their performance. There is a lack of knowledge on the impact of climate factors on the ecotoxicity of antimicrobials including TCS. The main objective of this study was to evaluate elevated temperature effect on triclosan toxicity to duckweed (*Lemna minor*) and its recovery potential. *L. minor* were grown in TCS-contaminated growth medium (1–400 µg L<sup>-1</sup>) under different temperature regimes (23°C and 27°C). After 7 days of TCS exposure, *L. minor* fronds were transferred to uncontaminated growth media for 7 days recovery. *L. minor* morphological indicators (dry weight, frond area), relative growth rate, biochemical indicators (enzyme activity and metabolites) as well as oxidative stress damage (lipid peroxidation) were measured.

### **2.02.P-Th105 Water contaminations by persistent toxic substances and responses of phytoplankton community in the Geum River Estuary, South Korea**



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A comprehensive understanding of the plankton ecosystem with water pollution has been limited considering changes due to freshwater discharge and the temporary influx of terrestrial substances. This study investigated the response of phytoplankton community to water contamination by persistent toxic substances (PTSs) and nutrients in an estuary affected by an artificial dam over one year. The distribution of PTSs, including 15 polycyclic aromatic hydrocarbons, 6 alkylphenols, and 8 metal(loid)s, along with nutrients, exhibited relatively high concentrations with irregular temporal fluctuations in the inner estuary. However, a few PTSs concentrations in the estuary exceeded the environmental quality guidelines of South Korea and the Canadian Council of Ministers of the Environment. The water quality parameters in the Geum River Estuary exhibit typical patterns seen in mid-latitude estuaries, with phosphorus playing a significant role as a limiting nutrient. During winter and spring, phytoplankton communities showed good ecological quality, with an average of 28 species and a density of 1750 cells L<sup>-1</sup>. In contrast, during summer, there was a significant increase in the density of freshwater species (max 45,000 cells L<sup>-1</sup>). These community were categorized into three seasonal groups, featuring dominant taxa like blue-green algae and diatoms. Temperature and nutrient levels were the principal factors influencing phytoplankton community, while PTSs had a minor impact. Overall, phytoplankton community displayed strong seasonal variation, mainly influenced by freshwater input and nutrient availability.

### **2.02.P-Th106 The Microscopic world: a comparison of different techniques for quantifying plankton**

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Plankton communities are integral to freshwater ecosystems. However, due to their small size, very short life cycles and dynamic ecological and environmental interactions, assessing these organisms on a community level can be very challenging. The presence of a stressor, such as a plant protection product (PPP), within the ecosystem creates an additional layer of complexity. Necessarily large datasets, acquired from samples collected over large temporal scale are required to decipher true direct and indirect effects against the dynamic natural changes to abundance. Traditionally, these communities are generally assessed using taxonomy techniques, however, there are new and emerging techniques for quantifying these microscopic organisms. When designing a study to investigate the impacts of a PPP on plankton, consideration must be given to the ecology and biology of these organisms, to ensure that appropriate methods are used for data collection. Freshwater phytoplankton cells vary in size by several orders of magnitude between species with any taxa recorded as colonies resulting in the biggest variation in size. Biovolume measurements can be used to adjust for this variation in size by calculating the total volume of each taxa and can be useful for assessing the functionality of individual species or groups, for assessing changes in morphology and for collecting data on algae taxa classed as colonies where it is difficult to distinguish individual cells. Abundance counts are able to show more subtle changes in the community structure, and these data are considered to be more standardized and statistically robust. Current standard practice for such measurements requires laborious microscopy techniques and expert taxonomic training, limiting the frequency and number of samples that can be processed. The development of metabarcoding techniques could provide an alternative, more efficient, method. Molecular analyses could be used in conjunction with microscopy identification, or as a complete separate analysis, to show variation in plankton communities as a large number of samples can be analyzed very quickly.

Here we present the benefits of reporting data from microscopy counts in terms of abundance or biovolume, and we introduce a comparison of traditional approaches against molecular data from a metabarcoding technique.

### **2.02.P-Th107 Incorporating macrophyte health measurements into higher tier risk assessments**

**Marie Brown, Zoe Leanne Parker-Crosse, Ellie Welch, Katie Smith, Hanna Samantha Schuster and Nadine S Taylor,** Cambridge Environmental Assessments (CEA), United Kingdom

Current guidelines recommend that at least 8 sensitive/vulnerable taxa, with acceptable minimum detectable difference (MDD) values, should be tested within a mesocosm study, however, there is little guidance on the selection of appropriate endpoints for macrophytes that will provide regulatory acceptable data. In addition, many assessment methods for macrophytes are considered subjective such as visual determination of chlorosis and necrosis. The lack of standardized methods and challenges associated with using this subjective and ordinal data to derive statistically meaningful endpoints can lead to questions over reliability and relevance in the risk assessment of PPPs. Despite this, these measures are useful in supporting the assessment of potential effects on macrophytes and as a weight of evidence approach for the derivation of suitable overall endpoints. The considerations for macrophyte studies should include ecological relevance as well as the practical implications of ensuring good growing conditions and appropriate assessment methods to achieve robust and reliable data. The unique sloped mesocosm employed at CEA allows a wider range of macrophytes to be planted within the mesocosm and methods have been optimised to assess macrophyte growth and health, increasing valid data collection throughout the study. The endpoints have been specifically developed for each species, in order to maximise the quality and quantity of data collected. These include height measurements, number of stems, number of nodes per cm, counts of leaves and flowers as well as visual assessments of aerial coverage (cm<sup>2</sup>) and percentage of chlorosis and necrosis. On completion of the test period, measurements of wet and dry

weight of each species are determined, however by measuring additional parameters throughout the study, this data can be used to contextualise the wet/dry weight results, assess short term effects and recovery, and determine the overall community effect of the test item.

By sharing our considerations for macrophyte endpoint evaluation in mesocosm studies we aim to highlight the relevance of mesocosm studies, particularly within the future of risk assessment for macrophyte communities. We will also demonstrate the sloped mesocosm design as a reference study in order to consistently derive more reliable and robust population and community level endpoints, using macrophytes as an example.

**2.02.P-Th108 Are TKTD Models for Algae and Macrophytes Protective in a Community Context – a Simulation Study**  
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Toxicokinetic-toxicodynamic (TKTD) models are more and more used as a tier 2 tool to address the time-variable exposure of aquatic organisms resulting from entries of plant protection products into edge-of-field surface waters. If successfully validated, the models can be used to predict the effects of many more complex exposure time series, predicted by regulatory exposure models (e.g. FOCUS step 3 or 4 models). However, if used as such and without coupling to a population model, the models still simulate laboratory tests and their outputs are the endpoints addressed in the corresponding tier 1 tests. For animals, these are organism level endpoints like survival, reproduction, development or growth. Under dynamic exposure, recovery of the organisms is possible (damage repair). In contrast to this, for algae and also for the standard test macrophyte *Lemna* sp., the standard tier 1 test assesses the inhibition of the population growth rate. Thus, dynamic exposure allows recovery of the population (ecological recovery). However, according to the EU Aquatic Guidance Document, Ecological Recovery can only be assessed in micro- or mesocosm studies, which means in a community context where species interactions can affect the recovery of a population. Because tier 2 studies provide the same type of endpoints as the related tier 1 test for algae and *Lemna* already population level endpoints are already used at tier 1, there is a dilemma with the general request that recovery of populations should only be assessed at tier 3. To analyse whether TKTD modelling for algae or *Lemna* is still protective in a community context, we used 2 different ecosystem models, Stolam and CASM. As worst-case scenario for recovery, we assumed that only one species is affected by a chemical (thus, all competitors are not affected). We simulated dynamic exposure profiles predicted to be regulatory acceptable by a TKTD model. Following current practice (Tier 1 RAC = ErC50 / 10), an acceptable profile was defined as the time series of PECs 10 times below the profile which resulted in 50 % inhibition of the growth rate in the simulated laboratory test. These profiles were compared to control runs and it was checked which effects the predicted acceptable exposure profile had on the different populations in the community. The poster will present first results.

**2.02.P-Th109 Aquatic primary producers in the prospective risk assessment for pesticides; some insights into considering the framework and connecting to the reality.**

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The environmental risk assessment (ERA) for pesticides follows a prospective tiered approach, starting with the most conservative and simple step (so-called tier 1). For aquatic primary producers, since 2015, the type of endpoint derived from ecotoxicological tests to use in ERA was changed; it is now the endpoint corresponding to 50% inhibition (EC50) of the growth rate (ErC50). Prior to 2015, it was the lowest EC50 based either on biomass (area under the curve- EbC50), yield (EyC50) or growth rate (ErC50). Our findings show that the use of the ErC50 while maintaining the default assessment factor (AF) of 10 clearly reduces the level of conservatism in the tier 1 RA compared to prior 2015; a minimum adequate level of protection is achieved now only in about 67% of cases versus 88% of cases prior to 2015. To restore the previous level of protection, an extra factor of minimum 2.4 could be used. However, it is questionable if this previous level of protection of 88% should be considered acceptable, since a sufficient protection level should theoretically be ensured for each individual substance. Besides that, the regulatory framework does not consider realistic conditions in agricultural landscapes where e.g. multiple stressors, pesticide mixtures and repeated application within and over the years occur. Ideally the tiered approach for primary producers should be appropriately calibrated towards such a realistic field situation since the population in the field is the actual entity to be protected. Some recent field monitoring studies of pesticides in surface waters have indicated concerns about their concentrations and ecological impacts. It is important to acknowledge these findings as well when questioning the protectiveness of the prospective risk assessment of pesticides.

**2.02.P-Th110 Protectiveness check of Tier-1 pesticide risk assessment for aquatic primary producers: learnings and possible next steps**

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In the last decade, the protectiveness of European aquatic risk assessment (RA) has been validated for insecticides, fungicides and herbicides by comparing the regulatory acceptable concentrations from Tier-1 (Tier-1 RAC) with those from mesocosm

studies (Tier-3 RAC), the latter representing the reference tier according to the EFSA aquatic guidance document (AGD). This resulted in several publications and conference publications. What makes herbicides and other substances with a herbicidal mode of action different from insecticides and fungicides, is the discussion which sublethal measurement endpoints to use in the risk assessment. The key aspect discussed in the validations of protectiveness performed so far is the impact of changing from biomass/yield related EC<sub>50</sub> values (E<sub>b/y</sub>C<sub>50</sub>) to growth rate related values (E<sub>r</sub>C<sub>50</sub>). Although E<sub>r</sub>C<sub>50</sub> values are in general slightly higher than E<sub>b/y</sub>C<sub>50</sub> values, it should be noted that only growth rate related figures allow a proper comparison of differences in sensitivity between species. By some authors these lower sensitivities are interpreted as a severe decrease of protectiveness within the RA. However, our work from the last two years demonstrated that only in 2 of 17 cases the change from E<sub>b/y</sub>C<sub>50</sub> to E<sub>r</sub>C<sub>50</sub> values resulted in a change of protectiveness for the substances involved. When discussing a potential loss of protectiveness in primary producer RAs, it is frequently not mentioned that the Tier-1 data requirements for herbicides regarding primary producers are usually exceeded, and therefore higher tier options are a next step. We have investigated higher-tier options for herbicides further and will present information on some complete primary producer related data sets. The newly presented data comparisons will also include additional data for *Myriophyllum spicatum* and other macrophyte species (e.g. *Glyceria maxima*) in order to study how protectiveness might change when adding data from additional macrophytes to the herbicide risk assessment. In addition for one compound we will add a modelling exercise to the comparison and compare Tier-1 RACs with Tier-3 and Tier-2C RACs.

## 2.02.P-Th111 The Influence of Abiotic Factors on the Distribution of Macrophytes in Small Water Bodies in Temperate Ecosystems

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Small water bodies (SWBs) have been shown to support both higher levels of gamma macrophyte richness and greater numbers of rare species than rivers or lakes, but they are facing unprecedented anthropogenic threats from habitat degradation, climate change and exposure to fertiliser and herbicide from nearby agricultural land. Little is known about how abiotic factors influence the distribution of macrophytes in SWBs and this makes it difficult to (i) design representative scientific investigations to understand how anthropogenic threats will impact macrophyte communities in SWBs, and (ii) target actions aimed at conserving and enhancing these ecosystems.

This is the first review to investigate the influence of abiotic factors on macrophyte distribution in SWBs. Thirteen different abiotic factors were studied: SWB type, SWB size, substrate size class, velocity, conductivity, depth, shading, surrounding land use type, hydroperiod, isolation, distance from the source, nutrient concentrations (phosphorus and nitrogen), and pH. Macrophytes were categorised by phylogeny into bryophytes and vascular plants, which were then subdivided by their morphology into emergent, rooted submerged, floating and submerged with floating leaves.

Results suggest that the impact of abiotic factors on macrophytes varies with macrophyte phylogeny and morphology. Both velocity and shading have a negative relationship with macrophyte richness and abundance for vascular plants, however a positive relationship with the richness and abundance of bryophytes, which favour shaded, fast flowing waters. Additionally, the substrate size class influences the distribution of emergent but not free-floating macrophytes, as emergent macrophytes require a fine sediment to anchor their roots. The proportion of land adjacent to the water body that is agricultural has a positive influence on the abundance and richness of vascular macrophytes, potentially because SWBs near agricultural land will have less shading from riparian vegetation and higher nutrient concentrations from fertiliser runoff.

The review provides information on how macrophyte communities vary across different SWBs, thus helping to target efforts from conservationists and policy makers and contextualising assessment of impacts from anthropogenic activities. The results also support the design of a long term mesocosm experiment, investigating the impact of herbicide exposure on macrophyte communities.

## 2.02.P-Th112 *Betula pendula* (Roth.) Seedlings Exposed to Polycyclic Aromatic Hydrocarbons: Insights into Plant Defense Mechanisms

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Polycyclic Aromatic Hydrocarbons (PAHs) represent a pervasive environmental challenge, originating from various anthropogenic activities such as industrial processes and combustion of fossil fuels. These persistent organic pollutants can have detrimental effects on plant physiology, disrupting cellular functions through the generation of reactive oxygen species (ROS). Understanding the impact of PAHs on *Betula pendula*, a keystone species, is essential for assessing the broader consequences of environmental contamination. This study investigates the relationship between PAH exposure and the activation of antioxidant enzymes in *Betula pendula* seedlings, focusing on the variability across different half-sib families (genetic groups). Seedlings from four half-sib families of *Betula pendula* were cultivated in hydroponic conditions using a nutrient medium supplemented with phenanthrene, pyrene, naphthalene, and fluoranthene, at varying concentrations (10, 100, and 200 µg L<sup>-1</sup>). This growth experiment spanned a period of 4 weeks. Following cultivation, leaves were harvested, and enzyme activities were measured spectrophotometrically. The assessment of antioxidant enzyme activity, including catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione-S-transferase (GST) serves as a molecular lens to explain the plant's defense mechanisms against PAH-induced oxidative stress.

Results showed that CAT enzyme exhibited increased activities in all half-sib families except those affected by phenanthrene, showing no impact or negative effects. POD enzyme displayed increased activity in one half-sib family, while three others experienced slowdown of its production or no impact. APX enzyme showed increased activity in one half-sib family but significantly decreased activity in three others. GST enzyme results divided half-sib families into two sections, with two showing increased activity and two with decreased activity.

In conclusion, the findings highlight that antioxidant enzyme activities in birch seedlings vary significantly among different half-sib families. Such a response suggests a genetic influence on the antioxidative defense mechanisms. This knowledge can inform the development of resilient plant varieties or the selection of suitable families for ecological restoration efforts in PAH-contaminated environments.

### **2.02.P-Th113 Multiplexed Algal Cytological Imaging (MACI) - A Novel Image-Based Phenotypic Profiling Assay for Screening Environmental Chemicals in Microalgae**

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High-throughput phenotypic profiling assays, which use high-content imaging to characterize changes in single-cell morphological feature data, have become popular in recent years for their ability to predict cellular targets and mechanisms of action (MoAs) for different chemicals and novel drugs. However, this approach has not yet been widely used in ecotoxicology due to the lack of studies and established methods for performing this kind of assay in environmentally relevant species, especially that of plant-type organisms. Here, we developed a novel image-based phenotypic profiling assay for *Raphidocelis subcapitata*, a toxicology and ecological model species, based on subcellular structures that are important and unique to unicellular microalgae (eg. chloroplast, nuclei, lipid droplets), which we coined as multiplexed algal cytological imaging (MACI). As a proof of concept, eight different herbicides and antibiotics with unique MoAs were exposed to *R. subcapitata* cells, and MACI was used to characterize their cellular impacts by measuring subtle changes in their morphological features, including metrics of area, shape, quantity, fluorescence intensity, and granularity of individual subcellular components. Based on clustering and neural network analyses, MACI was sensitive to the subtle changes in phenotype and was able to correctly delineate treated samples based on MoA. This study demonstrates that MACI offers a potentially quick and effective framework for screening environmental chemicals, and for characterizing their complex phenotypic responses, which can be used to determine their MoAs and identify their cellular targets in plant-type organisms.

### **2.02.P-Th114 Effects of Imidacloprid and Deltamethrin to aquatic macroinvertebrates using a microcosm approach**

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Pesticide contamination in aquatic ecosystems is a growing global concern. Pesticides are widely used to meet the food demands of a growing population, with various types used to control pests depending on the crops grown. Rainfall, overspray, and runoff from agricultural fields can wash these pesticides into water bodies, posing documented risks to the environment. This concern extends beyond regulators and the public to the industries that develop and apply these chemicals. Insecticides, in particular, are extensively applied for pest control, with over 3000 registered in South Africa alone. Pesticides can migrate from their point of application into non-target environments, leading to potentially harmful effects. Limited studies in South Africa have linked pesticide contamination to toxic impacts on aquatic and terrestrial ecosystems, with trace amounts lingering in soil and water. This study examines two widely available insecticides, deltamethrin and imidacloprid, which are moderately hazardous. This study assessed their effects to aquatic macroinvertebrates and zooplankton communities under field-realistic conditions using a microcosm approach. A microcosm is an artificially constructed test system that simulates parts of natural ecosystems. This study consisted of a 16 week exposure period where these pesticides were applied at week 0, 2, 4 and 6 and then left to see if recovery of the systems would occur. Macroinvertebrates were collected from the sediment, pebble baskets, plants and water column at week 0, 2, 4, 8, 12 and 16, while zooplankton were collected every 2 weeks from the water column. The results indicated that at higher concentrations these communities are severely impacted and that some organisms can tolerate these compounds and thrive. Even though these compounds break down rather quickly due to photolysis and cannot be quantified in the water and sediment the effects could still be seen after a long period of time.

### **2.02.P-Th115 Amoxicillin at Environmentally Relevant Concentrations Affects the Reproduction and Survival of the Littoral Cladoceran *Simocephalus punctatus*. A Life Table Study.**

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Amoxicillin (AMX) is classified as an emerging pollutant. This  $\beta$ -Lactam antibiotic is used to treat and prevent bacterial infections in humans and livestock. This drug is discharged into aquatic systems through treated and untreated wastewater, hospital effluents, and the disposal of expired drugs. Currently, AMX is detected in aquatic environments in concentrations ranging from ng to  $\mu\text{g L}^{-1}$  in different regions worldwide; nevertheless, the harmful effects that could produce this drug in aquatic biota are scarce. Cladocerans are used as test organisms to assess the effects of pollutants in aquatic environments. *Simocephalus punctatus* is a daphnid distributed in freshwater bodies in the coastal zone. In this study, in a life table assay, *S. punctatus* was exposed to amoxicillin (0.1, 1, 10, and 100  $\mu\text{g L}^{-1}$ ) for 21 days, starting from neonates. Daily, the released progeny (once reproduction started) and the adults' survival were recorded; the conditions of neonates were also observed. Reproductive responses were used to determine the age of first reproduction, the number of clutches, and the progeny per

brood; moreover, the life table parameters GRR, Ro, G, Ex, and r were calculated. Results were analyzed with the Bootstrap method, one-way ANOVA, and *post hoc* Tukey's test. Amoxicillin adversely affected the reproduction and survival of *S. punctatus*; additionally, in the concentration of 100 µg L<sup>-1</sup>, affected and dead neonates were observed (average of 4.16 and 3.96, respectively). All the life table parameters decreased significantly with the increase of amoxicillin concentration; the values of Ro ranged from 27.4 to 20.4 neonate female<sup>-1</sup>, while the population increase rate r varied from 0.32 to 0.29 neonate female<sup>-1</sup> day<sup>-1</sup>. The life expectancy (Ex) also diminishes from 18.3 to 17 days. The drug AMX, at environmentally relevant concentrations, produced detrimental effects on *S. punctatus*, warning about the potential impact of this drug in aquatic organisms, specifically on Cladocerans, which are an essential link in the energy transfer to high trophic levels in aquatic environments.

### **2.02.P-Th116 Screening of wheat lines for biological nitrification inhibition in hydroponics using a novel high-throughput experimental platform**

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The increasing use of nitrogen fertilizers in modern, intensified agricultural soils has resulted in higher nitrification rates, contributing to the disruption of the nitrogen cycle in soil. Approximately 50-70% of the nitrogen fertilizer used is lost to the environment through nitrate (NO<sub>3</sub><sup>-</sup>) leaching and gaseous nitrogen emissions before crop plants can uptake it. In the context of a climate change emergency and growing population, it is essential to find solutions to make agriculture more nitrogen efficient by improving nitrogen use efficiency (NUE) of crop plants. One such mitigation technique is crop plants with the ability to release biological nitrification inhibitor (BNI) compounds. This approach has been proposed to tackle N-emissions and NO<sub>3</sub><sup>-</sup> leaks, benefiting both agriculture and the environment.

The aim of this study is to employ a high-throughput bioreporter nitrification inhibition assay on root exudates of various double haploid wheat lines and its parental lines grown in hydroponics, to screen for their ability to inhibit ammonia-oxidizers. The bioreporter is a recombinant strain of the ammonia-oxidizing bacterium *Nitrosomonas europaea*, containing a bacterial luciferase gene (*luxAB*). The four parental lines have been further studied using the bioreporter at different incubation time points with the respective root exudates. Additionally, molecular analysis, nitrite measurements, and cell counting using the same bioreporter will be conducted to complement and gain a better understanding of limitedly studied BNI compounds.

We observe the crossed double haploid lines to exhibit BNI capabilities similar to the parental lines. When assessing the inhibition rates over time across the four parental lines, the results suggest the potential release of different BNI compounds with diverse modes of action. However, notable variability is observed among the biological replicates which needs to be further evaluated. The study further analyses the mode of action of the BNI compounds and reaffirms the efficacy of the proposed whole-cell bioreporter as a valuable screening tool for crop plants with BNI characteristics.

### **2.02.P-Th117 Challenges With Ecotoxicity Testing of Surfactants: The Impact of Water Hardness on Toxicity to Algae**

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Lithium salts of monocarboxylic acids are widely used chemicals, including as lubricants in the automotive industry and hydraulic fluids, and as such, they have the potential to exhibit substantial exposure to surface waters. However, the substances are difficult to test in aquatic toxicity studies. Due to the manufacturing method, many of these substances are considered as Unknown or Variable Composition, Complex Reaction Products or Biological materials (UVCB). Also, the substances have a soap structure, and thus form stable colloidal dispersions in water, and are surfactants with limited water solubility, which consequently limits their bioavailability.

The properties of surfactants pose challenges in ecotoxicity testing when attempting to conform to required regulatory testing approaches. It is recognised that water chemistry such as pH, hardness, or dissolved organic carbon (DOC), influences the availability of surfactants to organisms. In natural surface waters, the hardness typically exceeds levels found in standard algal growth test media. The United States Environmental Protection Agency (US EPA) recommends evaluating the effects of chemicals in both standard and a modified algal growth medium with increased hardness to ensure a relevant assessment of their effects in receiving waters. However, under the EU Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation non-standard testing approaches, such as changes to water chemistry, are now recommended to be avoided due to concerns that water hardness influences the bioavailability of the substances.

To address the concerns about the influence of water hardness on toxicity to algae, algal growth inhibition studies with two lithium salts of monocarboxylic acids substances were conducted following OECD 201 protocol at both standard (24 mg/L CaCO<sub>3</sub>) and increased (150 mg/L CaCO<sub>3</sub>) water hardness levels.

The results revealed no significant differences in toxicity of both tested substances at standard and increased test medium hardness, indicating that the standard testing approaches are sufficiently protective for algae.

These findings provide valuable insights into the impact of water hardness on the toxicity of surfactants, enhancing our understanding of their environmental behaviour and guiding future risk assessments.

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## **2.02.P-Th118 Physiological Effects of Suspended Sediments to Macroalgae Using Pulse-Amplitude Modulation (PAM) Fluorometry**

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Suspended sediments, often found in high concentrations in the water columns due to anthropogenic activities such as aquaculture, dredging, mining, and urban development, have adverse effects on various organisms in marine ecosystems. This study aimed to assess the biological response of suspended sediments on macroalgae and to enhance bioassays using imaging-pulse amplitude modulation (PAM) fluorometry to evaluate photosynthetic performance. Ecological effects on suspended sediments were tested with various species of macroalgae including nori (*Porphyra tenera*), kombu (*Laminaria japonica*), and five-ribbed kelp (*Costaria costata*). Exposure concentrations ranged from control to 10.0 g L<sup>-1</sup> of suspended sediment. Imaging-PAM fluorometry was employed to measure various endpoints: Fo, Fm, Y(II), Y(NPQ), Y(NO), qL, qN, qP, and ETR. The results revealed that nori and kombu exhibited decreased Fo with increasing suspended sediment concentrations. Notably, nori, with its thin thallus thickness, appeared to be most sensitive to suspended sediments among the species studied. The sensitivity to suspended sediment followed the order: nori > kombu > five-ribbed kelp. This highlights the higher sensitivity of red algae (nori) compared to brown algae (kombu and five-ribbed kelp) towards suspended sediments. This study confirms the impact of suspended sediments on the PSII performance of various macroalgae species. Exposure to suspended sediments in marine environments can potentially disrupt marine ecosystems by reducing food sources for upper trophic levels. These findings provide important insights for estimating suspended sediment criteria in marine ecosystems.

## **2.03 Assessing and Predicting the Impact of Chemical Pollution on Marine Mammals: Challenges to Be Overcome, Future Prospects, and Conservation Strategies**

### **2.03.T-01 How to detect impact of multiple anthropogenic stressors in Mediterranean cetaceans: the first application of exposomics**

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Concern is growing regarding hazards to Mediterranean cetaceans that simultaneously face concurrent exposure to multiple stressors, such as marine litter, climate change, as well bioaccumulation of contaminants. The main objective of this study was to apply for the first time a multi-diagnostic approach to demonstrate the use of exposomics combined with gene expression analysis for assessing the susceptibility of multiple stressors in an iconic whale species (*Balaenoptera physalus*) from the largest Specially Protected Areas of Mediterranean Importance: the Pelagos Sanctuary. Skin and blubber biopsies of fin whale were collected from the Mediterranean Sea (n=17), and from the pristine area of the Sea of Cortez (Mexico; n=11). **Exposome profile of skin biopsies fin whale:** In blubber (GC-HRMS), 58 substances were confirmed with authentic standards (Level 1), and 120 were annotated (Level 2). In total, 41 substances were quantified, including polychlorinated biphenyls (PCBs), organochlorine pesticides, polychlorinated dibenzofurans and polybrominated diphenyl ethers. A multivariate model significantly explained the variation in chemical exposures, with levels of PCBs higher in the samples from the Mediterranean than the Sea of Cortez. Several phthalate plasticizers were also detected by GC, and often higher in Mediterranean samples. A total of 386 compounds detected in skin by LC-HRMS were annotated, of which 113 in ESI+ mode (10 Level 1) and 273 in ESI- mode (25 Level 1). Of these, 39 compounds which belonged to a priority list of emerging contaminants, ranging from industrial chemicals, pharmaceuticals, biocides, plasticizers, bisphenols, PFAS, and UV-filters. The tobacco alkaloid nicotine, and its isomer anabasine, were detected in most of the Mediterranean samples. **Correlation between transcriptomic and exposome data in Mediterranean fin whale:** The correlation of 17 marker genes and exposomic data was investigated through a partial correlation analysis using the Spearman's coefficient. Interestingly, the levels of the plastic additives monoethyl phthalate (R<sup>2</sup>=0.73), monomethyl phthalate (R<sup>2</sup>=0.81) were found positively correlated with PPAR $\alpha$  expression; a positive correlation was observed with Aspirin as well (R<sup>2</sup>=0.70). In conclusion, we show a successful first application of chemical exposomics combined with gene expression analysis in cetaceans inhabiting the fragile and highly anthropized ecosystem of the Mediterranean sea in comparison to Sea of Cortez.

### **2.03.T-02 Associations Between Dietary Fatty Acid Tracers and Blubber PCB Concentrations in Eastern Beaufort Sea Belugas**

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The Eastern Beaufort Sea (EBS) beluga population, an important traditional food source for Inuvialuit communities in the Northwest Territories, Canada, represents a prominent Arctic odontocete species with strong associations to sea ice and varying habitat preferences based on size, sex, and reproductive status. Arctic ecosystems are undergoing rapid climate-associated change and a potential shift in diet from mainly Arctic Cod to Capelin could affect the body condition of individuals as these prey species are found at different depths and habitats in the Beaufort Sea. However, it is unclear how this climate-induced prey shift may impact the animal's exposure to environmental contaminants. The main goal of this study was to investigate the relationships between EBS beluga diet (estimated through fatty acid (FA) dietary markers) and blubber polychlorinated biphenyl (PCB) concentrations over a 22-year period. EBS beluga blubber samples ( $n = 166$ ) were collected during traditional Inuvialuit harvests in the Mackenzie Estuary area (Northwest Territories, Canada) between 1995 and 2017. Specific objectives were to assess: 1) the temporal trends of PCBs in EBS beluga blubber, 2) the relationship between specific FA dietary markers (20:1n-7, 20:1n-9, 20:4n-6, 20:5n-3, 22:1n-11 and 22:6n-3) as well as principal components derived from FA signatures and blubber PCB concentrations, and 3) the impact of male size class (small or large) on PCB levels and diet. We found that blubber  $\Sigma$ PCB concentrations in EBS belugas increased slightly between 2003 and 2017 for smaller EBS belugas. Moreover, several monounsaturated FAs (20:1n-7, 20:1n-9, 22:1n-11) were positively correlated with  $\Sigma$ PCB concentrations for both small and large males, whereas certain polyunsaturated FAs (20:4n-6 and 22:6n-3) were negatively correlated with  $\Sigma$ PCB concentrations for both size classes. We also observed that principal components derived from FA signatures were correlated with blubber  $\Sigma$ PCB concentrations. These results suggest that dietary markers may be valuable predictors of PCB exposure in EBS belugas, provided that body condition must also be considered as an important confounding factor. Understanding the ramifications of these dietary changes on EBS beluga exposure to PCBs and other bioaccumulative chemicals underscores the necessity for ongoing monitoring with a focus on the interconnectedness between environmental factors, pollution and marine mammal health in the face of climate change.

### 2.03.T-03 Fatty Acid Carbon Isotopes as a New Approach to Assess POP and Hg Accumulation among Marine Mammals and through Food Webs in the Arctic

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Top predators in the Arctic show among the highest concentrations globally of major contaminants of concern, including persistent organic pollutants (POPs) and Hg. To trace these contaminants among predators and through food webs, researchers have commonly used bulk stable isotopes (SI) of nitrogen and carbon. Yet, bulk isotope approaches have notable drawbacks, mostly from spatial variation in baseline signals and overlapping isotope values of different dietary resources. Here, we instead analyzed fatty acid carbon isotopes ( $\delta^{13}\text{C}$ -FA) and explored their potential to assess 1) interspecific variation in POPs among marine mammals (killer whale [*Orcinus orca*], narwhal [*Monodon monoceros*], long-finned pilot whale [*Globicephala melas*], and polar bear [*Ursus maritimus*]) in East Greenland and 2) biomagnification of Hg in a Cumberland Sound food web from zooplankton to Greenland sharks (*Somniosus microcephalus*). Several  $\delta^{13}\text{C}$ -FA values varied widely among species, while others were not significantly different. For example,  $\delta^{13}\text{C}$  values of the long-chain polyunsaturated FA 22:6n3 were highest in Greenland shark in Cumberland Sound and lowest in zooplankton, and also higher in East Greenland polar bear and killer whale than pilot whale and narwhal. In contrast, the  $\delta^{13}\text{C}$  values of monounsaturated FAs 20:1 and 22:1 in Cumberland Sound food webs did not differ among species, but were higher in East Greenland pilot whale and killer whale compared to narwhal and polar bear. Thus, these FAs may reflect spatial variation in  $\delta^{13}\text{C}$ -FAs as pilot whale and killer whale that only feed in the Arctic seasonally. Results from generalized linear models testing the influence of  $\delta^{13}\text{C}$ -FA values on contaminants relative to bulk isotopes and FAs will be presented. Preliminary results from the Cumberland Sound food web show that bulk  $\delta^{15}\text{N}$  better explains Hg concentrations than  $\delta^{13}\text{C}$ -22:6n3. However,  $\delta^{13}\text{C}$ -22:6n3 values explain more variation in PCB-153 than bulk  $\delta^{15}\text{N}$  among East Greenland predators. As such,  $\delta^{13}\text{C}$ -FAs may provide unique insights into the flow of dietary lipids and lipophilic contaminants across food webs, although bulk SI approaches may still better explain contaminant accumulation for proteinophilic contaminants, such as Hg. Yet, further testing of  $\delta^{13}\text{C}$ -FAs in other marine food webs system is warranted to confirm these findings.

### 2.03.T-04 Contaminant associated health effects revealed through a non-invasive metabolomics platform in at-risk killer whales in the Northeastern Pacific

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The Endangered Southern Resident killer whale (SRKW; *Orcinus orca*) population, consisting of only 73 individuals in the Northeastern Pacific, continues to experience a decline and is failing to recover due to a combination of stressors including reduced prey availability, noise and disturbance, and contaminants. Endocrine-disrupting contaminants including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs) are exceptionally high in the tissues of this population, which places them among the most contaminated marine mammals in the world. The ability to characterize contaminant concentrations and trends, as well as assess the health of SKRW, has been

constrained by legal and ethical constraints. Here, we measured contaminant concentrations in Resident killer whale fecal samples and applied a non-invasive targeted metabolomics platform to assess the health of SRKW in relation to contaminants. This characterization of contaminants and health in SRKW was strengthened through a comparative approach with Northern Resident killer whales (NRKW), a population that feeds on similar prey and is experiencing growth. Average fecal contaminant concentrations of PCBs, PBDEs, OCPs, HBCDD, APs and Hg were significantly higher (up to 5.5-fold) in SRKWs compared to NRKW. Average percent lipid was lower in SRKW compared to NRKW. SRKW were feeding more coastally and in closer proximity to urban sources compared to NRKWs, which is consistent with their elevated contaminant concentrations. A total of 238 metabolites were quantified in killer whale fecal samples. Compared to the NRKWs, SRKW had 24 metabolites that were upregulated (10% of metabolites quantified) and 21 that were downregulated (8.8%). Several of these altered metabolites correlated with contaminant levels in these individuals. Ongoing metabolomics data analysis will identify the metabolic pathways associated with contaminant exposure and biological variables (e.g. feeding ecology, % lipid) in these at-risk killer whale populations. This noninvasive approach using a fecal metabolomic platform serves as an effective tool to assess the effects of contaminants on the health of free-ranging Resident killer whales. Results of this study are providing much needed information on the current levels and trends of priority contaminants in these at risk killer whale populations and the associated impacts on their health.

### **2.03.T-05 A Framework for Developing Harmonized Environmental Quality Guidelines for Persistent Bioaccumulative Chemicals for the Protection of Marine Mammals and Their Habitat**

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Many jurisdictions have well established protocols to define environmental quality guidelines (EQGs) as contaminant sediment or water concentrations deemed to be protective for fish and benthos and, to a lesser extent, concentrations in prey for the protection of fish-eating birds and mammals. These have often been developed and applied separately, resulting in non-harmonized EQGs and, to the best of our knowledge, do not include consideration of long-lived apex predators, especially marine mammals. Here we outline a flexible EQG framework developed for persistent bioaccumulative (PB) chemicals that includes consideration of the complex life history of marine mammals that enables development of harmonized EQGs for sediment, water and prey to protect members of marine mammal populations deemed to be at greatest risk of adverse health effects. The framework includes two main pathways that can be used to calculate EQGs: A top-down approach starting from a toxicity reference value (TRV) for mammals that uses measured or modeled biomagnification and bioaccumulation factors, and trophic magnification factors to calculate EQGs for diet, water and sediment that align with the TRV; And a bottom-up approach that uses chemical fate and food web biomagnification models to calculate EQGs aligned with the chosen TRV. When available, empirical data are used to validate results. In this presentation we apply the framework to the case of PCBs in Canadian waters using fish-eating killer whales, beluga whales and Arctic ringed seals as representative species to be protected. Resulting EQGs calculated by either pathway yield EQGs for ΣPCBs that are lower than ones defined by the protocols currently used and show that killer whale populations are at greatest risk of adverse health effects from ambient ΣPCB concentrations. The flexibility of the framework enables its broader application to other marine mammals and less data-rich PB chemicals.

### **2.03.P Assessing and Predicting the Impact of Chemical Pollution on Marine Mammals: Challenges to Be Overcome, Future Prospects, and Conservation Strategies**

#### **2.03.P-Mo104 Can Amino Acid Nitrogen Isotope Analysis Provide New Insight into POP Biomagnification in North Atlantic Killer Whales?**

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Biomagnification is a key characteristic used to identify persistent organic pollutants (POPs). To evaluate trophic position (TP) for assessing POP biomagnification, nitrogen stable isotopes are measured in whole (bulk) tissues or organisms. Yet,  $\delta^{15}\text{N}_{\text{Bulk}}$  shows variability at the base ('source') of food webs that may confound assessments of TP and biomagnification. Instead, the  $\delta^{15}\text{N}$  of individual amino acids (AAs) are increasingly used to estimate TP, as taking the ratio of 'trophic' AA(s) relative to 'source' AA(s) may reduce confounding source variation. Yet, the extent of 'trophic' or 'source' AA increases with TP appears more variable among species than first thought, particularly for high TP species. Here, we explore  $\delta^{15}\text{N}_{\text{AA}}$  relative to other tracers to provide insight into TP and POP biomagnification in top predator killer whales in Greenland and Norway. Those in Greenland show higher  $\delta^{15}\text{N}_{\text{Bulk}}$ , suggesting that they feed at a higher TP compared to those in Norway. Killer whales in Greenland similarly show higher  $\delta^{15}\text{N}_{\text{AA}}$  of 'trophic' AAs than in Norway, but this pattern is also evident for 'source' AAs. 'Source' AAs may differ due to spatial variation in baseline  $\delta^{15}\text{N}$ , yet non-negligible trophic discrimination of 'source' AAs in high TP organisms may also contribute. Indeed, while the difference in  $\delta^{15}\text{N}_{\text{Glx}}$ , an established 'trophic' AA, between Greenland and Norway is  $\sim 3.7\%$ , an attempt to correct for 'source' variation by comparing instead the difference between Glx and the established 'source' AA, Phe, i.e.,  $\delta^{15}\text{N}_{\text{Glu-Phe}}$ , gives just  $\sim 0.2\%$  difference between sites. Either TP differences between these killer whales is not as large as suggested by  $\delta^{15}\text{N}_{\text{Bulk}}$  or typical approaches for  $\delta^{15}\text{N}_{\text{AA}}$  do not suffice for this and possibly



other high TP marine species. Concentrations of recalcitrant POPs are 10-fold higher in Greenland than Norway killer whales, consistent with  $\delta^{15}\text{N}_{\text{Bulk}}$  and diet estimates, but not with  $\delta^{15}\text{N}_{\text{Glx-Phe}}$ . As POP correlations with  $\delta^{15}\text{N}_{\text{Bulk}}$  are stronger than with  $\delta^{15}\text{N}_{\text{Glx-Phe}}$ , 'source' correction with Phe may remove not only 'source' but also 'trophic' variation. Thus, killer whale  $\delta^{15}\text{N}_{\text{AA}}$  show qualitatively but not quantitatively similar patterns of 'trophic' and 'source' AAs as found for other species, and typical approaches to estimate TP using  $\delta^{15}\text{N}_{\text{AA}}$  give unreasonable results that do not well explain POP variation. Basic studies on AA discrimination in high TP species and through marine food webs are needed to further evaluate this new approach.

### 2.03.P-Mo105 Unveiling Contaminant Exposure Patterns in Cetaceans from Madeira Island: Insights into Phthalates and Fatty Acid Markers

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Plastic additives, such as phthalates, are pervasive pollutants in aquatic environments, with concerning impacts on the health of marine life and the integrity of ecological systems. Monitoring key indicator species, such as cetaceans (e.g., dolphins and whales), can provide valuable information on the state and vulnerability of marine ecosystems. To investigate the interconnections among trophic niches (epipelagic vs. mesopelagic), contaminant levels, and individual health conditions, fatty acid profiles and phthalates were studied in blubber samples from two delphinids (short-finned pilot whale – *Globicephala macrorhynchus*; common bottlenose dolphin – *Tursiops truncatus*) on a remote island system. A multivariate study of chosen dietary fatty acids indicated niche segregation between the two species. An innovative method was developed and applied to detect and quantify phthalates in fresh cetacean blubber from biopsy samples. Out of the seven phthalates analyzed, the most encountered were di-n-butylphthalate (DBP), diethyl phthalate (DEP), and bis(2-ethylhexyl) phthalate (DEHP), with DEHP having the highest concentration ( $4697.34 \pm 113.45$  ng/g) in a bottlenose dolphin. Phthalate concentrations differed between the two species (Mean  $\sum$  PAEs:  $947.56 \pm 1558.34$  ng/g in bottlenose dolphin,  $229.98 \pm 158.86$  ng/g in pilot whale), with bottlenose dolphins mainly affected by higher concentrations of DEHP, and pilot whales by DEP and DBP. Health biomarkers indicated potential physiological challenges in pilot whales, although fatty acid profiles also revealed notable metabolic variations between the two species. No significant differences were observed in phthalate levels across ecological or biological groups, seasons, or years. This pioneering study examines the prevalence of plastic additive contamination in free-ranging cetaceans off Madeira Island, emphasizing the intricate interplay between ecological niches and contaminant exposure. Vigilant monitoring of these chemicals and their potential impacts is crucial to evaluating wild populations' health, guiding conservation efforts, and safeguarding critical species and habitats.

### 2.03.P-Mo106 Legacy POPs contaminant variation among cetaceans inhabiting the historically contaminated Gulf of St Lawrence, Canada

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Historical contamination in the Gulf of St Lawrence, resulting from industrial discharges in the North American Great Lakes region, has led to the accumulation of persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs), particularly in marine mammals. However, the accumulation of POPs in cetaceans feeding in the Gulf and adjacent downstream waters remains poorly known, fifty years after their ban. Here, we quantify PCB and OC concentrations in six species ( $n = 48$ ) of cetaceans in the Gulf of St Lawrence from 2015 to 2022. Knowledge on differential exposures between cetacean species will be useful for more targeted conservation strategies in Eastern Canada.  $\sum$ PCB concentrations were highest in killer whales (*Orcinus orca*), at  $101.6 \pm 32.9$  mg/kg lw, followed by other toothed whale species, then baleen whale species, with lowest concentrations in humpback whales (*Megaptera novaeangliae*) at  $1.0 \pm 0.4$  mg/kg lw. For OCs, similar patterns were observed, with killer whales having by far the highest concentrations, followed by toothed whales then baleen whales. Previous diet analyses have estimated that killer whales feed on baleen whales which would explain their elevated levels. While PCB and OC concentrations are lower in humpback whales and dolphins than concentrations measured in the 1990s, these compounds remain extremely high and above thresholds for risks of reproductive failure in killer whales. Contaminant profiles showed that common dolphins (*Delphinus delphis*) and killer whales had a high percentage of highly-chlorinated PCBs, such as CB-138, -180, -183 and -187, compared to baleen whales, for which CB-49, -52, -101 and -110 were the dominant compounds. For OC profiles, common dolphins and killer whales showed the highest percentages of DDE and *trans*-nonachlor, while baleen whales showed higher percentages of hexachlorobenzene and DDT. Elevated DDT proportions in baleen whales could represent a recent exposure to DDTs, as DDT is still being used for pest control in some countries. This represents the first study to quantify PCBs and OCs in such a broad range of cetacean species in the Gulf of St Lawrence. Future efforts will attempt to assess the impact of the species' feeding habits (through correlations with stable isotopes and fatty acids as tracers), to provide a more comprehensive understanding of contaminant accumulation among cetaceans inhabiting this historically contaminated area.

### **2.03.P-Mo107 Identification of potential impacts of an elevated and long-term exposure to POPs on the endangered beluga (*Delphinapterus leucas*) population from the St. Lawrence Estuary using transcriptomics**

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The St. Lawrence Estuary (SLE) beluga whale (*Delphinapterus leucas*) population in Canada is endangered. It has been postulated that exposure to elevated concentrations of polychlorinated biphenyls (PCBs), chlorinated pesticides, polybrominated diphenyl ethers (PBDEs), and emerging halogenated flame retardants (HFR) might be contributing to the decline of this population. Concentrations of PCBs, chlorinated pesticides and emerging HFRs in SLE male beluga blubber were shown to correlate with skin transcript levels of genes coding for nuclear receptors and proteins involved in the regulation of thyroid and steroid hormones as well as the metabolism of xenobiotics. A growing number of studies investigating contaminant exposure-related effects in cetaceans rely on “omics” methods such as transcriptomics, as these approaches require small amounts of tissue and are amenable to multiple analyses, yielding a maximum of biological information. To develop new transcriptomic health assessment tools to evaluate and understand the biological effects of contaminants in the endangered SLE beluga population, we compared transcriptomic skin profiles between adult male SLE belugas and Eastern Beaufort Sea (EBS) belugas (reference population). Being exposed to dramatically different levels of contaminants, the comparison of transcript profiles will allow to shed some light on specific genes and pathways affected by the exposure to organic contaminants. Total RNAs were isolated from skin samples of adult male belugas from the SLE ( $n = 35$ ) and EBS ( $n = 29$ ) and PCBs, PBDEs and emerging flame retardants were measured from their blubber. A total of 3,438 genes were upregulated, and 1,005 were downregulated in SLE belugas compared to EBS belugas. Among the metabolic pathways involved, several may be the results of epigenetic changes related to differences of exposure levels to PCBs, PBDEs and hexabromobenzene between these populations (e.g., regulation to reactive oxygen species, structural constituent of chromatin, cellular response to chemical and organic substances, immune system). These results represent a major advance in the identification and understanding of the impacts that long-term exposure to high levels of POPs have on the SLE beluga population. However, it remains difficult to know whether these observed effects remain specific to the skin, or whether they are an indication of a deeper disruption affecting the entire organism.

### **2.03.P-Mo108 Assessment of Polycyclic Aromatic Hydrocarbon (PAH) Exposure, Sources, and Maternal Transfer in Threatened Killer Whales (*Orcinus orca*) of the Northeastern Pacific Ocean (Canada)**

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The northeastern Pacific (NEP) Ocean spans the coast of British Columbia, Canada, and is considered impacted by anthropogenic activities including oil pipeline developments, maritime fossil fuel tanker traffic, industrial chemical effluents, urban wastewater discharges, and forest fires. Such events may expose surrounding marine environments to toxic polycyclic aromatic hydrocarbons (PAHs) and impact critical habitats of threatened killer whales (*Orcinus orca*). We analyzed skeletal muscle and liver samples from stranded Bigg's (Transient) killer whales and endangered Southern Resident killer whales (SRKWs) for PAH contamination using low-resolution mass spectrometry (LRMS). C3-phenanthrenes/anthracenes (mean: 632 ng/g lw), C4-dibenzothiophenes (mean: 334 ng/g lw), and C4-phenanthrenes/anthracenes (mean: 248 ng/g lw) presented the highest concentration across all tissue samples. Diagnostic ratios indicated pyrogenic-sourced burdens for Bigg's killer whales and petrogenic-sourced contamination for SRKWs; differences between ecotypes may be attributed to habitat range, prey selection, and metabolism. A SRKW mother-fetus skeletal muscle pair provided evidence of PAH maternal transfer; low molecular weight compounds C3-fluorenes, dibenzothiophene, and naphthalene showed efficient and preferential exposure to the fetus. This indicates *in-utero* exposure of PAH-contamination to the fetus. Our results show that hydrocarbon-related anthropogenic activities are negatively impacting these top predators; preliminary data found here can be used to improve oil spill and other PAH pollution management and regulation efforts, and inform policy to conserve killer whale NEP habitats.

### **2.03.P-Mo109 Trace element concentrations in dolphins of south-east Australia show elevated mercury concentrations for bottlenose dolphins**

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Among Cetaceans, trace elements have been linked to serious health issues such as neurological and reproductive disorders, immunosuppression, and in some cases leading to mortality.

In the south-east of Australia, trace element toxicology has traditionally focused on sediments and water, with biological studies centered on fish, mollusks, and penguins. Earlier investigations revealed exceptionally high mercury concentrations in stranded Burrunan dolphins (*Tursiops australis*) sampled between 2004-2008, surpassing those in well-known compromised

waterways like the Mediterranean. This raised questions about whether other dolphins in the region share similar trace element burdens or if this phenomenon is species-specific.

This study presents trace element concentrations in three dolphin species: the Burrunan dolphin, short-beaked common dolphin (*Delphinus delphis*), and the common bottlenose dolphin (*Tursiops truncatus*). Species-specific differences emerged, with both Burrunan and common bottlenose dolphins exhibiting higher hepatic ( $641 \pm 1,405$  mg/kg,  $730 \pm 655$  mg/kg, respectively) and renal ( $56 \pm 46$  mg/kg,  $60 \pm 33$  mg/kg, respectively) mercury (Hg) levels than short-beaked common dolphins (hepatic;  $144 \pm 110$  mg/kg, renal;  $17 \pm 14$  mg/kg). This was found to be significant for renal tissues ( $p < 0.05$ ).

Conversely, short-beaked common dolphins displayed significantly higher concentrations of arsenic (As) in both hepatic ( $2.2 \pm 1.2$  mg/kg) and renal tissues ( $1.5 \pm 0.8$  mg/kg), as well as renal cadmium (Cd) compared to Burrunan (hepatic As;  $0.9 \pm 0.8$  mg/kg, renal As;  $0.4 \pm 0.3$  mg/kg, renal Cd;  $25 \pm 28$  mg/kg) and common bottlenose (hepatic As;  $0.92 \pm 0.4$  mg/kg, renal As;  $0.53 \pm 0.5$  mg/kg, renal Cd;  $13 \pm 7.5$  mg/kg) dolphins.

Burrunan and common bottlenose dolphins in this region exhibit among the highest mercury (Hg) concentrations globally, with only three studies reporting higher hepatic Hg in dolphins. Additionally, mercury-to-selenium (Hg:Se) ratios raise concerns, as almost all adults surpass the 1:1 molar ratio threshold for hepatic toxicity concentrations.

The elevated Hg levels observed across species in the region, combined with the accumulation of Hg and Cd, pose potential concerns. Hg:Se ratios above one for most adults suggest a diminished ability to mitigate the toxic effects of mercury. This study emphasizes the necessity of ongoing monitoring and understanding trace element burdens in marine mammals to preserve both these species and the broader ecosystem.

### **2.03.P-Mo110 Twenty years of temporal trend assessment for regulatory contaminants in common dolphins (*Delphinus delphis*) from French Atlantic waters**

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Marine mammals may act as sentinel species for monitoring long-term trends in chemical contaminant exposure of the marine environment. Indeed, they are long-lived species that often occupy high trophic positions, making these species integrative and reliable indicators of environmental pollutant pressure, especially for bioaccumulating and biomagnifying contaminants. Here, the temporal trends of regulatory contaminants were analysed in the most abundant and hence representative cetacean species of French Atlantic waters, the common dolphin (*Delphinus delphis*), over a nearly twenty-years period (2000-2019). Moreover, this species is subject to high rates of bycatch in fishing nets in the area, which adds to the chemical pressure.

The aim of this assessment was to evaluate the temporal variations in contaminant levels in common dolphins in order to set the base for the development of an indicator of chemical pollution in marine predators from French waters. Three toxic trace elements (Hg, Cd and Pb) and seven classes of persistent organic pollutants (POPs: polychlorinated biphenyls, dichlorodiphenyltrichloroethanes, polybrominated diphenyl ethers, hexachlorobenzene, hexachlorocyclohexanes, chlordanes and mirex) were studied using dynamic linear models, for a total of 201 dolphins stranded along the French Atlantic coasts. Temporal trends were estimated taking into account the age, the gender and stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) measured in individuals, as proxies for their feeding ecology. The results showed that Pb concentrations in dolphins decreased over time, probably following the lead petrol regulation after the 2000s. In contrast, increasing concentrations were observed for total Hg and Cd. For POPs, all classes analysed showed decreasing trends, with the exception of hexachlorobenzene. As molecules regulated by the Stockholm Convention, these results are in line with what would be expected and with most of the literature for POPs. The identification of temporal variations in concentrations in these sentinel species validates the use of these trends (i.e. relative values of concentrations) as a contamination indicator of the marine environment.

### **2.03.P-Mo111 Relationships among Mercury Concentrations, Sea Surface Temperature, and Survival in Steller Sea Lion Pups in the Aleutian Islands, Alaska**

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Although many Steller sea lion (*Eumetopias jubatus*) metapopulations within the endangered western Distinct Population Segment have stabilized or begun to recover after a dramatic population decline in the 1970–90's, some rookeries in the western and central Aleutian Islands continue to decline. To examine mercury/contaminants as potentially contributing to these divergent trends in abundance, we examined spatio-temporal patterns in mercury concentration and direct effects on survival probabilities. Lanugo (natal fur) samples collected from pups on Agattu Island (western Aleutian Islands) over 8 breeding seasons between 2011-2022 ( $n=389$ ) showed a broad range of [THg] ( $2.55\text{--}73.74$   $\mu\text{g/g dw}$ ). We identified a significant doubling in median [THg] in the lanugo of pups over this brief period from  $8.0$   $\mu\text{g/g}$  in 2011 to  $19.7$   $\mu\text{g/g}$  in 2022 (Kruskal-Wallis  $H=54.56$ ,  $p < 0.001$ ). The proportion of pups sampled on Agattu Island with lanugo [THg] above  $20$   $\mu\text{g/g}$  THg (threshold

of concern) increased more than twofold from 21% in 2011 to 48% in 2022. To elucidate potential correlations between [THg] in lanugo and survival probabilities, we used [THg] from those pups permanently marked at birth and subsequently resighted as an individual-based covariate in a mark-resight framework. During the study period, localized September sea surface temperature fluctuated in the western Aleutian Islands with the lowest average temperatures seen early in the decade between 2010-2012 ( $8.9 \pm 0.2$  °C), peak fall temperatures over 11°C found in 2015 and 2018, and mean fall temperatures of  $9.8 \pm 0.2$  °C between 2020-2022. Preliminary results indicate that lanugo [THg] above 10 µg/g was weakly positively correlated with pup and yearling sea lion survival over the study period in some metapopulations, though larger sample sizes are needed to elucidate region- and sex-specific effects with greater certainty. We discuss potential mechanisms for these results and outline ongoing efforts to maximize the utility of available data to examine the potential effects of this and other contaminants on this endangered top predator.

### 2.03.P-Mo112 True seals as bioindicators of emerging pollution in the Northeast Atlantic Ocean

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Amidst the ongoing global energy transition, demand for Critical Raw Materials (CRMs) employed in strategic sectors such as renewable energy and digital technologies is surging. However, knowledge gaps exist regarding the trophic transfer and toxicological effects of CRMs on marine biota. True seals being long-lived and coastal predators that are widely distributed across the Northeast Atlantic, serve as ideal bioindicators for assessing pollution levels within marine food webs.

This study quantifies the concentrations of 21 CRMs in muscle and liver tissues from six seal species: the harp seal *Pagophilus groenlandicus*, hooded seal *Cystophora cristata*, ringed seal *Pusa hispida* and bearded seal *Erignatus barbatus* from European Arctic waters, and the grey seal *Halichoerus grypus* and harbour seal *Phoca vitulina* from the Southern North Sea and the Channel (OSPAR Region II). Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), we detected CRMs, including REEs, Li, V, Mn, Co, Ni, Cu, Y, Ta, Ga, Sn, Sb, Tl, and U in 103 muscle and 110 liver samples from adult seals. Principal Component Analysis (PCA) and Redundancy Analysis (RDA) were utilized to encapsulate CRM concentration variability and to identify influential factors such as species, sampling area, and tissue type.

Findings reveal the presence of all CRMs across each species and tissue, with concentrations varying from as low as 0.00004 mg.kg<sup>-1</sup> dw (U in muscle) to 161mg.kg<sup>-1</sup> dw (Cu in liver). Tissue type emerged as a primary factor influencing CRM variability, with the liver being notably more contaminated, except for Li, which was more concentrated in muscle ( $F = 255$ ,  $p < 0.01$ ). PCA results indicates that REEs primarily contributed to the variability along PC1(39%). Seals from the Greenland Sea exhibited higher concentrations of REEs (Mean  $\sum$ REEs: 0.27mg.kg<sup>-1</sup> dw) compared to their North Sea counterparts ( $\sum$ REEs: 0.07mg.kg<sup>-1</sup> dw). Additionally, significant interregional and interspecific variability influenced by sex was observed, with males presenting higher CRM levels ( $F = 4.716$ ,  $p = 0.001$ ).

The study highlights tissue specificity and potential intersex trophic differences as major contributors to CRM variability, laying the groundwork for developing pollution indicators. These insights are pivotal for the initiatives of the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention) to prevent and regulate marine pollution by emergent contaminants.

### 2.03.P-Mo113 Contaminant Burden of Stranded Sentinels - The Case Studies for Lulu and Spike

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Although recent years have seen conventions and regulations implemented to protect ecosystems from pollution, the effect of e.g., legacy contaminants remain a threat to wildlife. Heavy metals, such as mercury, continue to be released from both natural and anthropogenic sources and are ubiquitously present in the environment. They bioaccumulate in marine wildlife and biomagnify through the foodweb, with the potential to cause irreversible damage to organism health. Top predators, such as some marine mammal species, are thus at increased risk of contamination. It has been accepted that the prevalence and persistence of pollutants in marine ecosystems can be approximated from pollutant levels in marine mammal tissues, making them ecosystem sentinels. An understanding of the contaminant burden and health status of these animals may provide a more holistic insight into the ocean's status.

We measured the concentrations of 9 analytes (mercury, methylmercury, arsenic, cadmium, copper, lead, nickel, selenium, and zinc) in tissue samples from 15 marine mammal species that stranded along the Scottish coastline between 2015 and 2022 using ICP-MS and TDA-AAS. The samples were provided by the Scottish Marine Animal Stranding Scheme (SMASS). Our results indicate an organ-dependent trend for some of the analytes, although animals of the same species accumulated vastly different amounts of contaminants. Alongside overall trends, we will showcase two animals, Spike the bottlenose dolphin and Lulu the Orca. Spike's samples were used to test various high-throughput methods we adapted for mercury speciation

assessment. As bottlenose dolphins are under protection, insights into their heavy metal contamination and overall health are vital for their conservation. Lulu's samples were also analysed for e.g., PCBs by other researchers, and can thus be used to highlight the life-time contaminant accumulation.

Our findings have highlighted that while overall trends in contaminant accumulation in trop marine predators can be observed, more focus must be placed on understanding individuals and processes involved. Vulnerability factors must be better quantified in order to guide future protection and conservation measures.

#### **2.04.A Bridging the Gap between Exposure, Ecotoxicology and Ecology – Identifying and Regulating the Impact of Chemical Pollution on Biodiversity**

##### **2.04.A.T-01 Indirect Antimicrobial Effects in Aquatic Food Webs – Using Heterotrophic Pathways as a Model**

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Many registered chemicals enter freshwater ecosystems, potentially affecting ecosystem functions. One crucial function is the decomposition of leaf litter, contributing substantially to the energy supply in aquatic food webs. This function is achieved by microbial decomposers and macroinvertebrate detritivores. Antimicrobially active chemicals can affect leaf-associated decomposers, specifically bacteria and fungi, leading to indirect effects on higher trophic levels by altering litter quality for detritivores. These indirect effects are currently not considered in environmental risk assessment, although potentially propagating along the food web and beyond the borders of aquatic ecosystems. Changes in the leaf-associated microbial community composition have been associated with alterations in leaf consumption across major detritivore taxa. For instance, amphipods and caddisfly larvae avoided fungicide-exposed leaf material when control leaves were provided as alternatives. This avoidance behavior resulted from the dominance of unpalatable fungal species within the leaf-associated microbial community. Contrary effects have been observed with substances having an antibiotic mode of action. In this case, *G. fossarum* preferred leaf litter exposed to antibiotics over control leaf litter, likely due to the competitive release of fungi resulting from antibiotic effects on bacteria, leading to higher fungal growth and diversity. Persistent consumption of low-quality leaf litter can have lasting effects on detritivore physiology. Consequently, these effects may lead to further consequences along the food chain, including alterations in fine particulate organic matter production and reduced secondary production, which has been observed in multiple studies. Furthermore, fungicides and antibiotics can induce indirect effects on detritivore physiology by altering the gut microflora, reducing efficiency in nutrient and energy assimilation. This reduced efficiency could impact detritivore growth and reproduction, potentially having long-term adverse effects on leaf litter decomposition. Finally, the effects of fungicides taken up by trees on leaf litter quality and microbial interactions have not been extensively studied, but initial findings suggest indirect effects on detritivores through altered leaf quality. Considering these indirect effects during chemical testing will help safeguard ecological integrity within aquatic ecosystems and beyond their boundaries.

##### **2.04.A.T-02 The mesocosm HeMHAS: A novel non-forced system to integrate the ecosystems' structural and functional changes due to contaminants in a multispecies scenario**

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Although the forced exposure system has shown its usefulness for establishing the relationship between exposure and effects, it lacks ecological relevance for motile organisms (as many organisms can move out from contamination) and when contamination is not homogeneously distributed. To address these limitations, Mesocosm HeMHAS (Heterogeneous Multi-Habitat Assay System), a novel system, was constructed to assess the structural and functional changes in a community resulting from exposure to contaminants, while considering the complex ecological scenarios of heterogeneous landscapes. Copper concentrations (0, 1, 10, 100, and 250 µg/L) were chosen as a case study due to their reported presence in estuarine and marine ecosystems. Species belonging to different trophic levels: fish (*Sparus aurata*), shrimps (*Palaemon varians*) and three different classes of marine microalgae (*Isochrysis galbana*, *Nannochloropsis gaditana* and *Tetraselmis chuii*) were used as test organisms. Motile fish and shrimp were used to assess contamination-driven habitat selection, while sessile microalgae represented toxic effects on non-mobile species. Other species as *Artemia* sp., macroalgae and macrophytes were also introduced into the system as environmental enrichment. All species were distributed across five interconnected mesocosm compartments for 24 h, with three replications. Results revealed that fish and shrimps selected habitats where copper concentrations were not higher than 1 µg/L (24 h-AC<sub>50</sub>: 4.88 µg/L) and 50 µg/L (24 h-AC<sub>50</sub>: 136.58 µg/L), respectively. The lower avoidance of shrimp was linked to a balance between predation risk and exposure to contaminants. The study suggested that species' habitat selection against contaminants may vary based on interspecies interactions, potentially jeopardizing population persistence. Among the non-mobile organisms, the growth of *I. galbana* was more sensitive (growth reduction of 50%). The nutrient uptake was only rapid for ammonium. The mesocosm HeMHAS should be considered as a complementary tool in ecological risk assessment studies as it provides complex scenarios of interconnected disturbed habitats for higher ecological relevance and a broader assessment of stressor effects. From a Stress Ecology perspective, mesocosm HeMHAS

identifies how contaminants, besides their continuous toxicity, stress organisms and disturb habitat selection, affecting the spatial distribution of biodiversity.

#### **2.04.A.T-03 Progress in the Application of Non-Invasive Targeted Environmental DNA (eDNA) Methods for Enhanced Confidence in Risk Assessments and Evaluation of Mitigation and Remediation Effectiveness**

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Environmental DNA (eDNA), genetic material released by organisms into the environment, can provide rapid, non-destructive, accurate population distribution and biodiversity information. Within the context of ecotoxicology, eDNA methods can provide critical field data related to complex mixture and chronic, sublethal exposure impacts. Moreover, mitigation and remediation effectiveness can be readily assessed without disturbing at-risk species or their habitats. However, poorly performing tools hinder broad adoption of eDNA-based detection methods, due in part to their associated high false negatives and false positives that can impair effective management decision-making. We therefore addressed workflow issues associated with targeted eDNA assay design to help improve user confidence in the approach. We focused on targeted quantitative real time polymerase chain reaction (qPCR)-based eDNA assays as a highly accessible and mature technology. Yet, several challenges remain to analyze environmental samples for eDNA. These include effective identification of unique regions of target taxon mitochondrial DNA conducive to high quality assay design. This is best accomplished with full mitogenome sequences of target and relevant non-target taxa. However, there is a significant gap in publicly available resources, including mitogenome sequences, especially from multiple voucher specimens living in each environment. It is often extremely difficult to obtain these specimens, let alone obtain high quality DNA from them. To address this, we created a protocol for amplifying small amounts of DNA suitable for whole genome shotgun sequencing. The resulting sequencing reads can then be assembled using *mtGrasp*, a utility we developed for reference-grade mitogenome sequence assembly. The full mitogenome sequences from target and non-target taxa are processed by *unikseq*, a utility we developed for the identification of unique DNA sequence regions for specific qPCR primer and probe design within a target taxon compared to non-target taxa. Primers and probes are empirically validated to ensure they meet or exceed the qPCR-based assay performance criteria as established by the recently released CSA W219:23 national Canadian standard. Through use-case examples, we demonstrate the efficacy of this approach for application of eDNA methods in environmental surveys. *mtGrasp* and *unikseq* are freely available as software repositories at <https://github.com/bcgsc>.

#### **2.04.A.T-04 Micro-pollutant Biotransformation in Field-collected Fish: Implications for Species-specific Sensitivity to Chemical Pollution**

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Among the multiple biological processes that are triggered in response to chemical exposure, biotransformation pathways are key in supporting detoxification and the elimination of xenobiotics. However, while biotransformation-related genes are often conserved among species, their inducibility and translation to enzymatic activity in relation to species sensitivity to pollution remains understudied. Environmental and anthropogenic factors such as pollution level may also alter biotransformation activity and the ability of species to cope with chemical exposure. Thus, in the present study, fish species that are representatives of Swiss biodiversity assemblages were collected in watercourses with different levels of pollution and surrounded by different land use. Liver S9 enzymatic fractions were isolated and comparative assessments were conducted to assess enzymatic activity and biotransformation rates of different pharmaceuticals and pesticides. Among species, brown trout (*Salmo trutta*) displayed the highest activity for the enzymes CYP1A and Glutathione-S-transferase, particularly in sites with high levels of anthropogenic influence. However, the invasive species pumpkinseed (*Lepomis gibbosus*) presented significant activity of other biotransformation enzymes, like CYP3A4, and the highest intrinsic clearance and biotransformation product formation of most of the micro-pollutants tested. On the other hand, bottom-dwelling species, like the European bullhead (*Cottus gobio*) and the common barbel (*Barbus barbus*), displayed the lowest enzymatic activity and micro-pollutant biotransformation. These observations underline biotransformation processes as major indicators of species sensitivity, making them an important consideration when evaluating the role of chemical pollution in altering the biodiversity of aquatic ecosystems.

#### **2.04.B Bridging the Gap between Exposure, Ecotoxicology and Ecology – Identifying and Regulating the Impact of Chemical Pollution on Biodiversity**

##### **2.04.B.T-01 Synthetic Chemicals as a Cause of Biodiversity Loss**

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Industrial and agricultural discharges cause European rivers to be exposed to mixtures of synthetic chemicals. It is unclear to what extent biodiversity is being affected by the cocktail effect of chemicals. The current mandatory Water Framework Directive's (WFD) monitoring schemes fail to accurately quantify if pollution results in insufficient ecological status, where

biological parameters for assessing ecological status are not related to chemical exposure. Although knowledge is lacking, the EU Biodiversity Strategy for 2030 acknowledges pollution as a key driver of biodiversity loss. In this project, the limited knowledge of how biodiversity is affected by chemicals will be addressed through an eco-epidemiology study in which ecology and ecotoxicology are integrated. Modelled mixture toxicity pressure from 1760 synthetic compounds will be linked to available macro-invertebrate field data present in European rivers. The results will reveal to what degree and which synthetic chemicals are responsible for biodiversity loss, which will be used to help improve the implementation of WFD. The ambition is to strengthen the bridge between scientific evidence and water management policies to help create a toxic-free environment.

#### **2.04.B.T-02 From Ecotoxicology to Ecology: Changes in Macroinvertebrate Communities Along a Toxicity Gradient**

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Water pollution is one of the major stressors to freshwater ecosystems with significant inputs of nutrients and micropollutants such as trace metals, industrial chemicals, pesticides and pharmaceuticals. Considering the large number of chemicals in the environment, current standard chemical monitoring methods are limited for technical and economic reasons. In addition, complex mixtures with interactive effects impede the environmental risk assessment. Ecotoxicological tests with water and sediment samples could overcome these limitations by assessing the overall toxicity of a sample and providing further indications through specific mode-of-action testing. Thus, so-called effect-based methods are increasingly used in water and sediment quality monitoring. However, the ecological relevance of ecotoxicological effects observed in these methods is rarely evaluated. To address this gap, we used data from the DECIDE project, which aims to develop an ecotoxicological assessment system for rivers and streams complementary to the current monitoring practices of the European Water Framework Directive. At 30 sites in central Germany, a comprehensive set of *in vivo* and *in vitro* methods was tested for their informative value and practicality. These ecotoxicological investigations were complemented by an assessment of the benthic macroinvertebrates, local river habitat structure and supporting physicochemical quality components. By linking ecotoxicological and ecological data, we investigated the changes in the benthic macroinvertebrate community along toxicity gradients. More specifically, we aimed to evaluate the ecological relevance of toxicities derived by effect-based methods and the potential of macroinvertebrate communities as pollutant-specific indicators.

We like to thank the German Federal Environmental Foundation for funding the DECIDE project (AZ 35663/01).

#### **2.04.B.T-03 Pesticide Fate in Understudied Freshwater Vertebrates: the Case of a Threatened Terrapin (*Emys orbicularis*) in Several Wetland Regions of France**

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Pollution is one of the major threats to freshwater ecosystems and remains understudied in wetlands, especially agricultural pollution. Chronic exposure to water pollution from increasing plant protection product applications is expected to play a role in biodiversity decline in these ecosystems, but little is known about the fate and effects of pesticides in wild populations, notably reptiles. Freshwater turtles, especially, are longlived species with high spatial fidelity and suffer from local contamination. We worked on the European pond turtle (*Emys orbicularis*) a species with high potential for pesticide exposure. From 2018 to 2023, we conducted longitudinal and transversal studies and sampled 15 populations of *E. orbicularis* in three regions of France. We obtained 719 blood samples from 568 individuals and we assessed the levels of 40 pesticides in LC-MS/MS after a single step of purification-extraction by acetonitrile. We also evaluated the contamination pathways by taking water and soil samples, passive samplers, and sampling crayfish, one of the main prey of *E. orbicularis*. During the three-year longitudinal study on two populations in the Camargue, we detected 24 pesticides among 29, on 408 blood samples. We showed that the site of capture and its hydrology was one of the main factors explaining pesticide occurrences and levels, alongside the day of capture, with an increase of both of these variables across the season of capture. However, the frequency of detection was low except for bentazone, an herbicide widely used. Thanks to the longitudinal monitoring, we recaptured 116 individuals over two or three years, and we showed that bentazone levels varied at the intra-individual level. Regarding the transversal study, we have already analyzed 102 blood samples from 5 populations of the Camargue. We confirmed the effect of the site of capture as the main explicative factor. We showed that the frequency of bentazone detection was related to its concentration in the water. The samples from the 10 other populations will be processed at the time of the congress. Analyses are currently underway to measure the effects of pesticide exposure on various biological parameters that we hypothesize to be disrupted in populations living in the most contaminated sites. Together, our results suggest a rapid response of this species to environmental pesticide contamination and call for attention to bentazone, a widely and heavily used herbicide worldwide

#### **2.04.B.T-04 Effect of Pesticides on Soil Microbial Networks of Variable Trophic Diversity**

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The toxicity of pesticides to natural ecosystems is an ongoing concern, yet little is known about the ecotoxicological effects of these compounds on soil microorganisms and microbial food webs. Since microbial predator-prey interactions play an essential role in the soil ecosystem by modulating microbial diversity and nutrient distribution at various trophic levels, we aimed to assess the effect of two pesticides at the recommended application dose on microbial communities using a food-web approach. Microbial communities with increasing diversity of predators were assembled in gamma-sterilized soils: i. bacteria only (extracted from soil), ii. bacteria + selected bacterivorous protists and iii. bacteria + selected protists with different feeding modes and prey preferences. Systems were prepared in quintuplicate and sampled on days 0, 7, and 21 after pesticide addition. Functional aspects were addressed by substrate-induced respiration (SIR), microbial biomass, total N and C,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{PO}_4^{3-}$  analyses. The effects on microbial community composition and abundances of functional groups were assessed by Illumina 16S rRNA gene sequencing and qPCR of nitrogen-cycling bacteria, respectively.

Our results show a higher sensitivity of archaeal nitrifiers (as measured by *amoA* gene abundance) to pesticides compared to bacterial nitrifiers. However, at the recommended application dose, the effect was only observed in microcosms with higher trophic diversity. A consistent decrease in 16S rRNA gene abundance was observed in line with the increase in trophic diversity, while no direct effect due to pesticide exposition was observed. Nevertheless, indirect effects of pesticides on the microbial community composition were observed depending on the level of trophic diversity. These results provide insight into the soil microbial response to pesticide exposure and confirm the need for food-web assessments to avoid an underestimation of pesticide effects on soil microbial communities at recommended application doses.

#### **2.04.C Bridging the Gap between Exposure, Ecotoxicology and Ecology – Identifying and Regulating the Impact of Chemical Pollution on Biodiversity**

##### **2.04.C.T-01 Impacts of antimicrobial stressors on bottom-up regulation in aquatic macroinvertebrate food webs**

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In heterotrophic streams a substantial proportion of the energy budget is represented by organic matter of terrestrial origin, particularly leaves. Nutrients and energy bound in leaf litter is made accessible to the wider aquatic food web by microbial decomposers (bacterial communities and fungi) and shredding macroinvertebrates. Microorganisms can improve the nutritious quality of leaves for shredders by reducing leaf toughness and by concentrating essential elements (i.e., conditioning). Antimicrobial substances, which are released into streams via wastewater or runoff have the potential to modify the conditioning processes leading to bottom-up directed effects on shredding macroinvertebrates. However, consequences for species interactions translating to changes in the aquatic food web are unclear. Therefore, the main objective of this study was to investigate bottom-up directed effects by antimicrobial stressors in aquatic macroinvertebrate food webs. For this purpose, leaves were conditioned two weeks under exposure to 8 concentrations of an antibiotic (Ciprofloxacin) and a fungicide (Azoxystrobin), respectively, leading to alterations in the leaf-associated microbial community. After modification of the conditioning process, these leaves were provided to an extended macroinvertebrate food web in model stream ecosystems (16 x 3.6 m long flow through stream microcosm). The macroinvertebrate communities were only slightly indirectly affected by Ciprofloxacin while Azoxystrobin resulted in significant indirect impact on the composition of the macroinvertebrate communities with increasing concentrations. Analyses of stable isotopes and fatty acids are ongoing. Additional experiments possibly explain changes in heterotrophic food webs, such as respiration and feeding plasticity tests. It is expected that the combined results will provide new insights into the magnitude and mechanisms of these effects in heterotrophic food webs in terms of changes in feeding preferences, affected physiological fitness translating to altered trophic interactions and community structure. These insights could generate fundamental knowledge for an area that has received little or no attention in the past.

##### **2.04.C.T-02 Beyond the Crop: Assessing Insecticide Deposition and Ecological Risks for Non-target Arthropods in Flower Strips**

*Michael Peter Meissle, Research Division Agroecology and Environment, Agroscope, Switzerland*

While Plant Protection Products (PPPs) play a crucial role in modern agriculture, concerns about biodiversity loss exist, especially for non-target arthropods (NTAs). In Swiss agriculture, temporary flower strips are one government-promoted element to provide additional habitat and food supply for valued NTAs. When crops need to be sprayed with insecticides and other pesticides, however, arthropods in flower strips and other off-crop habitats may potentially be affected by PPP inputs through spray drift. The current off-crop risk assessment relies on models, assumptions, safety factors, and methods extrapolated from the in-crop situation, thus high uncertainties regarding actual exposure, effects on communities, and recovery of NTAs in off-crop habitats remain. The presented project aims at addressing those challenges for the habitat of flower strips. Specifically, we study drift deposition in the flower strip structure; we investigate toxic effects on NTAs in the field by simulating realistic drift scenarios; and we establish a vulnerability index for NTAs using existing toxicity databases combined with trait-based databases.



Field studies were conducted in Tänikon, Switzerland from 2021 to 2024 involving flower strips arranged in a plot design. The plant-dwelling NTA community within the flower strips was monitored using a suction sampler before and after application of acetamiprid insecticide. Preliminary data will be presented. The outcome of this multidisciplinary research, combining exposure measurements, effects data, and ecology in a realistic field setting, will inform risk assessment regulation and policy regarding promotion of flower strips and other non-crop habitats near field that may be subject to PPP applications.

#### **2.04.C.T-03 Indirect Effects of Pesticide Use on Farmland Birds: Lessons from 50 Years of Monitoring, with a Way Forward.**

*Julie Ann Ewald, Stephen J Moreby and Nicholas J Aebischer, Game & Wildlife Conservation Trust*

The Game & Wildlife Conservation Trust has monitored, through the Sussex Study, farmland biodiversity and pesticide applications on an area of the South Downs in southern England for over 50 years. The study was designed to monitor changes in grey partridge (*Perdix perdix*) numbers across a core 32 km<sup>2</sup> of mixed farmland. Monitoring includes not only grey partridge abundance and productivity, but also arable flora and invertebrates in the cereal crops, which provide food for grey partridges and other farmland birds (both as adults and at the chick stage). Pesticide use, on a field-by-field basis, has been monitored over the same time-frame. Over the 50 years, analysis of changes in food availability and pesticide use indicates a significant negative effect of insecticide use on the abundance of important groups of chick-food invertebrates, from an 8% to 35% decline when insecticides were used, controlling for cereal type, year, herbicides and fungicides. In the last 20 years, one farm on the study area was determined to recover grey partridges after breeding density declined from eight pairs per 100 ha to one pair per 100 ha from 1970 to 2003. Its efforts have been successful, resulting in an average density of 14 pairs per 100 ha over the past 15 years. We explore what the farm has accomplished, how farm management changed to achieve recovery, how pesticide use was restricted as part of this management, and the effects on both arable flora and invertebrates. The other farms on the study area have undertaken some conservation activities but at a lower level, allowing a comparison with a more “business as usual” approach. We compared pesticide use on the Sussex Study to national statistics from similar crops, to put the Sussex Study into perspective. On average across the Sussex Study herbicide and fungicide use was less intensive than nationally, while insecticide use was similar to use across the UK. This talk updates previous work on the long-term monitoring ongoing on the Sussex Study area and illustrates a way forward to help mitigate the indirect effects of pesticide use in a cereal-based ecosystem.

#### **2.04.C.T-04 Assessing In-Field Pesticide Effects Under European Regulation and Implications for Biodiversity**

*Stephan Brendel, Magali Solé, Steffen Matezki, Jeremias Martin Becker, Klaus Swarowsky, Sabine Duquesne, Silvia Pieper and Jörn Wogram, German Environment Agency (UBA), Germany*

The use of pesticides plays a major role in the biodiversity loss in agroecosystems and it was shown that the negative effects exerted by pesticides are persistent. Despite legal requirements that pesticides should not have unacceptable environmental effects, there is a lack of an adequate assessment regarding their impact on on-target terrestrial plants (NTTPs) and arthropods (NTAs) in agricultural fields. Such in-field areas are important, as they constitute a substantial proportion of the total area in typical agricultural landscapes. NTTP and NTA are the basic components of terrestrial food webs and play an integral role in terrestrial biodiversity. Thus, their decline can have cascading effects on consumer species, including farmland birds and mammals; these potential indirect effects via alteration of food webs are also largely ignored in the current environmental risk assessment.

Shortcomings lie within the guidance documents that do not adequately implement the requirements of the current legal framework. An in-field assessment for NTTP is missing and the implemented in-field assessment for NTA is inadequate. To overcome these shortcomings, we developed a readily applicable assessment scheme that allows to assess these risks; it is based on the currently available data required in regulations.

In order to bridge science and regulation, a workshop was held in July, 2023 at UBA in Dessau, Germany. The workshop's experts agreed on the necessity of adequately considering effects of in-field exposure of pesticides in risk assessments. While the proposed scheme was considered appropriate, it was acknowledged that more profound changes beyond pesticide regulation are needed for sustainable agriculture.

In conclusion, to protect biodiversity and to satisfy legal protection requirements, the risk assessment of NTTP and NTA must include an appropriate assessment of in-field effects and indirect effects via alteration of the food webs. In our contribution we highlight current gaps and present a simple assessment scheme to broadly consider the outlined deficiencies in risk assessment.

#### **2.04.C.T-05 Consideration of Environmental Risk Assessment for the protection of biodiversity under the EU Pesticides Regulation (EC) No. 1107/2009**

*Rachel Sharp, Domenica Auteri, Csaba Szentes, Alessio Ippolito and Fernando Alvarez, European Food Safety Authority (EFSA), Italy*

In the context of the EU regulation of pesticide plant protection products (EC No. 1107/2009) the potential impact of the active substance on biodiversity, and the ecosystem, including potential indirect effects via alteration of the food web (i.e., indirect effects), should be considered. Whilst a definition for biodiversity is given in Regulation EC No. 1107/2009, its operational definition in the context of a regulatory assessment of pesticides is ambiguous. Although various proposals have been made to

integrate indirect effects into the Environmental Risk Assessment (ERA) performed for pesticides currently, no harmonised and widely accepted approach is available. Nevertheless, the protection of biodiversity, via the assessment of indirect effects, have been highlighted to be a key area for improvement for the EU regulatory framework of pesticides. This presentation will use the case of the biodiversity assessment performed for the EU renewal of glyphosate to highlight key issues and make proposals for how this area of ERA may be further advanced. An update will also be given to what current, and near-future, EFSA activities will feed into such development.

#### **2.04.P Bridging the Gap between Exposure, Ecotoxicology and Ecology – Identifying and Regulating the Impact of Chemical Pollution on Biodiversity**

##### **2.04.P-Tu075 MeMo – Mesocosm Modelling – Building a bridge from higher tier data to ecosystem modeling**

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Owing to the complex interplay of the growing number of stressors affecting aquatic communities, it is becoming increasingly important to predict the potential effects as accurately as possible. Outdoor aquatic mesocosms have already been used in risk assessment for over two decades to investigate effects of stressors under more realistic conditions. In order to be able to utilize this comprehensive higher tier data even better and more efficiently in the future, this data might be complemented and extended via modeling e.g. by simulating multiple or time variable exposure scenarios by application of mechanistic effect models. Therefore, providing a solid data basis is essential. For this reason, the MeMo Environmental Database Plus (MeMo Umweltdatenbank<sup>PLUS</sup>) project was launched. The project, which is funded by the Hessen Agentur GmbH, focuses both on making historical data sets from mesocosm experiments usable for modeling and on generating new data sets meeting modeling requirements.

Within the project, a mesocosm study was performed in which classical sampling methodologies were characterized with regard to their representativeness and effectiveness. This was achieved, e.g., by fully sampling of macroinvertebrates in individual mesocosms following application of classical sampling methodologies. With this method, extrapolation factors can be determined from the classical trapping technique and the absolute numbers of organisms in the entire mesocosm. By applying the determined extrapolation factors, total abundance, which is needed for modeling purposes, can be calculated retrospectively from historical count data.

In a second phase, a follow-up mesocosm experiment assessing potential harmful effects on aquatic freshwater organisms of the veterinary pharmaceutical eprinomectin was carried out with extended chemical analysis. In addition to the classical abundance data, the community structure (frequencies of size classes of species) of macroinvertebrates was determined. In this way, it is possible to determine a potential influence on the biomass of individual taxa. This allows to document effects on the population in much greater detail. In future, these extensive ecosystem data sets allow a better understanding of effects at population and ecosystem level and can be used as a basis for various modeling approaches.

##### **2.04.P-Tu076 Predicting Bacterial Functional Responses From Water, Biofilm, and Sediments to Land Use-Derived Chemical Pollution at River Basin Level**

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Microbial organisms, especially bacterial communities, are key players in biochemical processes that sustain ecosystems. Furthermore, they are also responsible for degrading toxic chemicals and developing resistance against antimicrobial drugs, ultimately impacting human health. In the field of microbial ecotoxicology, there is not standard approach for assessing the effect of trace organic chemicals derived from human activities, such as pesticides, pharmaceuticals and personal care products, and industrial chemicals on bacterial communities. Therefore, the use of DNA- and RNA-sequencing-based methods is called upon to play a role defining new assessment frameworks. In the last decades, most studies have focused on assessing structural microbial responses to chemical pollution, including shifts in biodiversity, increase or decrease dissimilarities, and correlations to chemicals. Consequently, functional microbial assessment, such as the degradation of toxic chemicals and nutrient recycling processes, critical microbial functions, is often overlooked. In this study, environmental DNA (eDNA) was analysed from water, biofilm, and sediment samples collected in the River Aconcagua Basin (Central Chile) to evaluate: i) whether bacterial functions changed depending on the type of land uses, ii) identify potential bacterial functions affected or linked by chemical pollution, and iii) determine whether these changes are compartment-specific or affect the entire bacterial community. Our results suggest negative impacts on the degradation potential of organic chemicals in bacterial communities inhabiting stream and rivers affected by nutrients and antibiotics compared to reference conditions. Specifically, significant reductions in the degradation of hydrocarbons and aromatic compounds were determined in water, biofilm, and sediment samples. We predict changes in processes related to the nitrogen cycling, such as potential reduction of nitrogen fixation in

bacterial in water and biofilms in stream running through agricultural streams compared to reference conditions at a river basin level.

#### **2.04.P-Tu077 Multiple contaminants in complex communities: evaluating non-additive effects of multiple simultaneous stressors on biomass flux and ecosystem functioning**

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Biodiversity has declined as a result of numerous stressors, including chemicals. Conventional environmental risk assessment approaches tend to evaluate single stressors individually, with effects assessed at the individual and population scales. This overlooks the fact that multiple stressors impact multiple species interacting in complex communities, with indirect effects emerging at a community level. Understanding how multiple sublethal stressor effects combine is crucial to be able to predict and mitigate their impacts on biodiversity. By combining food web theory of interacting species with ecotoxicological theory of contaminant effects, we performed in-silico modelling experiments to assess how biomass and stability of a tri-trophic food chain respond to herbicide-like and pesticide-like contaminants. We applied these contaminant effects simultaneously across stressor intensity gradients, varying the trait rates and trophic levels targeted by the pesticide-like contaminant. We measured community biomass as an indicator of productivity and ecosystem functioning, investigated the distribution of biomass across the community (i.e. trophic level biomass), and calculated stability metrics. Using recent methods to distinguish between additive and multiple non-additive antagonistic and synergistic effects, we found responses in biomass and stability to be dominated by additive and antagonistic effects and no synergistic effects. We found that patterns of non-additivity in community and trophic level biomass varied depending on the trait and trophic level targeted by the pesticide-like contaminant. This study reveals how simple community structure and the modification of direct and indirect effects in a community by contaminants leads to diverse outcomes of multiple stressors on biomass and stability. This demonstrates the value of leveraging ecological community modelling techniques to evaluate the long-term indirect effects of multiple sublethal contaminants acting on multiple species on ecological functioning.

#### **2.04.P-Tu078 Evaluation of toxicological effects of fungicides used in vineyards agroecosystem using *Apis mellifera* as a model species**

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Agroecosystems provide habitats, food and water, for many species, but are areas where pesticides and fertilizers are widely used. Pesticides are the most important threat to pollinator insects that live in agroecosystems, especially due to their intensive use in agriculture. Many European regions are famous for wine production, with land covered by vineyards where the biodiversity is absent and to preserve wine production many pesticides are used. The European Directive 128/2009/CE and No 540/2011 regulate the use of pesticides in agroecosystems. Although many active principles have been banned, some substances such as those used in viticulture are already permitted. Breed bees and wild bees come in contact with the pesticide used in vineyards collecting the morning dew from vine leaves and using sugar from the grapes, especially during increasing periods of drought. Our study aimed to investigate the toxicological effects on honeybees, used as model species, of the commercial formulation of two fungicides: 1)Folpet-based and cymoxanil+copper-based, alone and in combination and the toxicological effects of 2)copper. Laboratory experiments with fungicides and copper and a 2 years beehive survey in two vineyards with different types of management (conventional and organic) during the use of this type of fungicide were carried out. A multi-biomarker approach was applied to test the neurotoxic effect (AChE, CaE), metabolic alterations (ALP), detoxification system (GST), efficiency of the immune system (LYS, proPO, PO and hemocyte count and genotoxicity (NA assay). The results of the laboratory experiment with Folpet-based, and cymoxanil+copper-based showed induction of GST activity in all treatments except the mixture at the lowest concentrations and a decrease in the ALP activity. LYS activity increased in the groups exposed to the Folpet-based fungicide, to the lower concentration of cymoxanil-copper-based and the mixtures. The pesticide interaction model showed that the mixture of the two fungicides had subadditive effects. The beehive monitoring showed no esterase inhibition during and after the treatment of the vineyards compared to the organic vineyard and metabolic alterations and effects on the immune system were found in bees sampled during the vineyard treatments. This study confirms the need to deeply investigate the sublethal effects of commercial pesticides, in particular those used in vineyards, to protect wild and farmed pollinators.

#### **2.04.P-Tu079 Differential Gene Expression of Freshwater Macroinvertebrates Exposed to Micropollutant Mixtures across the River Holtemme (Germany)**

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Chemical pollution is recognised as one of the major drivers influencing the ecological status of aquatic ecosystems. Micropollutants are organic chemicals occurring at trace environmental concentrations, including pesticides, pharmaceuticals, personal care products, and industrial chemicals among other, which have raised concern due to their negative impact on aquatic organisms. Some of these chemicals have known mode and mechanism of action (e.g., neonicotinoid insecticides

altering nicotinic acetylcholine receptors). Nevertheless, for many chemicals their mode of action is unknown. Besides, we still have a poor understanding of the combine effects when these chemicals co-occur in the aquatic environment. Aquatic benthic invertebrate fauna represents one of the Biological Quality Elements, which is used to determine the Ecological status of surface water bodies according to the EU Water Framework Directive. They are therefore extensively used as biological indicators to assess water quality. Nevertheless, a thorough understanding of the adverse biological consequences of micropollutant mixtures at the pathway level under real exposure conditions is still absent. There is not a standard approach defined to unveil such effects and most of the evidence available is based on single gene assessment. This study employs an RNA-based-sequencing (transcriptomic) approach on *Gammarus pulex* populations, shredder invertebrates, across a gradient of stations with increasing chemical pollution in the River Holtemme to address this gap. Non-targeted gene assessment reveals alterations in gene expression, with an increasing number of differentially expressed genes (DEGs) downstream of chemical pollutant influences. Notably, a station downstream of a wastewater plant exhibits up to 6014 DEGs compared to the reference site. Our findings indicate a significant upregulation of invertebrate DEGs downstream of wastewater treatment plants. Furthermore, a pathway enrichment analysis identifies over-represented gene classes in categories such as nervous system development, carcinogenesis, and stress response across all stations under micropollutant stress. Thus, our results link observed biological responses to specific chemical groups, shedding light on the intricate interplay of micropollutants and their impact on freshwater macroinvertebrates in freshwater ecosystems.

#### **2.04.P-Tu080 Investigating the relationship between contamination and coral reef condition in the Red Sea: An integrated assessment from bacteria to fish**

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Coral reefs are the most diverse marine ecosystems, harboring over one quarter of known marine life. They provide habitats and resources for numerous sedentary and mobile organisms, from small invertebrates to large fish. They also play a critical role in biogeochemical cycling and coastal protection, being essential drivers of economic activities. Coral reefs are, however, undergoing rapid changes due to the synergy of global and local stressors. One of the most detrimental threats resulting from anthropogenic activities is pollution, caused by the release of contaminants from different sources, such as treated or untreated municipal, industrial and agricultural wastes. Marine pollution can severely affect the biodiversity and the functioning of coral reef systems. However, comprehensive studies integrating contamination and biological information across multiple components of the reef biodiversity are scarce. Here, we present the preliminary findings of an inter-disciplinary project conducted in the Red Sea spanning three regions of contrasting anthropogenic pressure and including biodiversity (bacteria, corals, and fish), bioaccumulation patterns in fish, and environmental data, including inorganic and organic contaminants. Our dataset includes *in situ* data of reef benthic structure and fish density and biomass, molecular-based assessments of the metazoan and prokaryotic communities (18S and 16S, respectively) in reef sediments, bioaccumulation patterns in fish, and concentration of trace metals and metalloids, as well as targeted emerging contaminants (Personal Care Products; e.g., medicines, caffeine), used as indicators of human pressure. We predict that the concentration of contaminants mirrors the anthropogenic gradient and that the condition of the coral reefs (measured as hard coral cover, fish biomass, diversity, and composition of metazoan and prokaryotic communities) is affected by the increasing human pressure, i.e., the concentration of contaminants in the reef abiotic matrices (sediments and waters). This study is one of the few integrating multiple angles of the coral reef ecosystem following an inter-disciplinary approach and will contribute to elucidating the existing ranges of contaminants in Red Sea coral reefs and guide dedicated ecotoxicological studies and future regulations. The results of this project can also help managers implementing mitigation strategies to alleviate the effects of marine pollution in Red Sea coral reefs.

#### **2.04.P-Tu081 A complementary approach based on contaminant analysis, biomarker responses and behavioural performances to investigate the toxicological status of *Parus major* from Veneto region**

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Pesticides and other classes of anthropogenic contaminants can cause reversible alterations or permanent damage to avian species. Great tit (*Parus major*) is an important model species for environmental studies. Its aptitude for nesting in artificial boxes and territoriality can provide precise toxicological information on a given territory. In the last six years, several nest boxes for great tit were installed in different areas of Veneto region, characterised by different anthropogenic impacts. This study aimed to investigate the effects of environmental contaminants on great tit populations by a non-lethal approach integrating chemical analysis, biochemical/cellular responses, breeding and behavioural traits. In 2021 and 2022, biological materials such as blood, feathers, excreta and unhatched or abandoned eggs were collected from nestlings living in nest boxes installed in different areas of Veneto region. The study areas, subjected to different anthropogenic pressures, were wooded,

agricultural and urban environments. Per- and polyfluoroalkyl substances (PFAS) and heavy metal levels were determined on eggs and feathers, respectively and porphyrin concentrations were evaluated on excreta. A set of non-lethal biomarkers capable of providing different toxicological responses was performed on blood. Behavioural traits such as reactivity performances and fitness were also assessed. PFOS was the most abundant compound, and the long-chain perfluorinated carboxylic acids levels were agricultural < urban < wood. Cu was the most abundant metal in all areas and Hg had higher values in the urban area. Nestlings from urban and agricultural areas had higher genotoxic effects than this from wood. The H/L ratio was more severe in urban birds, which showed a physiological stress condition. Complement system activity was higher in nestlings from an agricultural area suggesting that these birds might be exposed to substances that act on innate immune system. The applied approach was proved to be a valuable tool for the ecotoxicological monitoring of great tit. It is fundamental to obtain a complete picture of the health status of this species and to investigate the environmental quality in which the studied species lives.

#### **2.04.P-Tu082 Assessment of multiple stressors of aquatic ecosystems using invasive *Corbicula* clams in Argentina**

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Multiple stressors shape populations, communities, and ecosystems. In freshwater ecosystems, chemical pollution, temperature changes, and parasites are common stressors, yet few studies have investigated them simultaneously. Invasive clams of the genus *Corbicula* are widespread in Argentina and can be used to assess their impacts. In 2021-2023, we collected *Corbicula largillierti* clams to assess chemical pollution by pesticides in an irrigation channel in the northern part of the country (Salta) and *Corbicula fluminea* congeners to assess urban pollution in the Río de la Plata Estuary (Buenos Aires). In Salta, we conducted 48-h exposure experiments to two concentrations of glyphosate (0.3 and 3 mg L<sup>-1</sup>), imidacloprid (20 and 200 µg L<sup>-1</sup>) and their mixture, and one control (total N=35). In Buenos Aires, we assessed 120 clams from two populations exposed to contrasting pollution conditions: Parque de los Niños strongly impacted by urban pollution and Punta Lara away from this influence. In all populations, we measured the effects of pollution on standard metabolic rate and several parameters of oxidative stress: concentrations of proteins, glycogen, reduced glutathione (GSH), thiobarbituric acid-reactive substances (TBARS), and glutathione S-Transferase (GST) activity. Additionally, in Buenos Aires, we studied the influence of temperature (12°C and 21°C) and *Chaetogaster limnaei* gill parasites (estimated prevalence and intensity following clam dissection) on the metabolic and cellular endpoints measured. The degree of exposure to urban effluents was assessed using detailed physicochemical datasets of each site from monitoring programs of the Argentine Ministry of Environment and Sustainable Development in both sites in Buenos Aires. This was complemented with direct assessment of metals (cadmium, copper, chromium, iron, nickel, lead, and zinc) in the gills, digestive gland, and mantle of the clams. We found that imidacloprid had a more pronounced effect compared to glyphosate on the respiration rates of *C. largillierti* clams in Salta, although additive effects on oxidative stress parameters were clear when both pesticides were present. Our analysis of Buenos Aires populations shows differential effects of environmental stressors on metabolism and oxidative stress that suggests a dependence of context. These findings highlight the use of *Corbicula* spp. clams and multiple biomarkers to enhance biomonitoring sensitivity of multiple stressors in aquatic ecosystems.

#### **2.04.P-Tu083 Using effect-based methods to assess chemical stress in old river restorations**

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Aquatic ecosystems are often altered by multiple stressors, including hydrologic and morphological degradation, high nutrient loading, and chemical pollution. More than 90% of German surface waters do not meet the objective of at least good ecological status of the Water Framework Directive. To improve freshwater habitats and thus the ecological status of water bodies, hydromorphological restoration has been increasingly implemented. However, follow-up assessments often show little to no improvement in ecological status even years after restoration measures have been implemented. The failure of many restoration projects can be attributed to the fact that not all relevant stressors were considered. These stressors include water pollution and inadequate sediment quality, which often receive too little attention compared to non-chemical stress. For this purpose, effect-based methods (EBMs) are recommended to gain a better insight into the impacts of pollutants and their underlying mechanisms. In this study, the impact of chemical stress on five restored streams, each with a restored and a nearby located non-restored section, was investigated using EBMs. All streams do not achieve good ecological status according to the EU-WFD. We aimed to determine whether there is a difference in the toxicity and chemical composition of water and sediment samples between restored and non-restored river sections, and if water pollution is a factor that causes the failure to achieve good ecological status.

In this study, we used a bioassay battery to evaluate the effects of water and sediment samples on organisms of different trophic levels. In addition, water samples were analyzed by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). To simplify the existing data set from the effect-based assessment and chemical monitoring, a principal component analysis (PCA) was used.

Using EBMs, high activity levels in water and sediment samples of restored streams were found. Differences in toxicity between restored and non-restored sections did not show a consistent trend, depending on the individual test. However, concerning chemical monitoring of water samples, non-restored and restored sections of the same stream were similar in their chemical composition. Accordingly, the present findings suggest that chemical pollution appears to be a non-negligible factor limiting restoration success and improvement of the ecological status of rivers.

#### **2.04.P-Tu084 Moving towards a better protection of terrestrial biodiversity – identify, compare, redefine**

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Chemical pollution is a planetary boundary threat and an important driver of biodiversity decline, which stands in contrast to the European vision to establish a non-toxic environment and put biodiversity on a path to recovery. Despite of this goal, the assessment and evaluation of biodiversity as such is not sufficiently covered in chemical legislation (e.g. REACH). Regulators require sound data to understand and quantify chemical pollution's impact on biodiversity at several scales and to be able to develop means to effectively protect it – something that is currently not available. Furthermore, today's chemical risk assessments are often very generic and only a small fraction of the marketed chemicals have been evaluated sufficiently regarding their (eco)toxicological properties or exposure scenarios. The HORIZON project TerraChem (Nov 2023 – Oct 2026) strives to provide novel and urgently needed monitoring data on the extent of chemical contamination in terrestrial food chains and the adverse effects of single contaminants and their mixtures at an organism level, as well as to develop new methodologies to assess their impact at an ecosystem level. This poster will display how these data can be used to improve the efficacy of current risk assessment practices and trigger further regulatory action by identifying priority substances, while also inspiring change within the political and regulatory frameworks.

#### **2.04.P-Tu085 Developing Environmental Scenarios for The Risk Assessment of Non-Target Organisms**

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One of the key aspects of the Environmental Risk Assessment (ERA) performed within the plant protection product authorisation process is that such assessments should be able to predict risks over large geographical areas (e.g., over an entire country, regulatory zone, or even over the entire EU). However, the nature and the magnitude of environmental risks are very much influenced by several biotic and abiotic factors, which are variable in space and time. Therefore, accounting for context-dependent factors is pivotal in developing future methods for ERA.

The concept of environmental scenarios is considered a pragmatic way for condensing the variability of environmental characteristics most likely to have an impact on environmental risks.

This poster will lay down the specific objectives of forthcoming work which will trace the pathway to define the environmental scenarios.

At first, an analysis of the European agricultural landscape will be carried out to identify clusters with similar characteristics. Later, high resolution mapping, gathering georeferenced information particularly on non-cropped landscape elements, such as field margins and other semi-natural areas, will be used to refine the initially defined clusters.

In a subsequent work, habitat and biological assemblages representative of the identified clusters in agro-ecosystem will be fully characterised via systematic literature reviews and field monitoring. Later, the vulnerability of such assemblages to plant protection products will be investigated once again via systematic literature reviews and via targeted experimental studies. The vulnerability assessment will then constitute a further layer in the clustering exercise before the definition of the final scenarios. Developing scenarios should serve for multiple purposes:

- Aligning the conditions used for the exposure assessment and the risk characterisation, incorporating spatial, temporal, and ecological aspects that have not been routinely considered so far.
- Defining baseline conditions which in turn can be used for specific assessments as well as input for the derivation of Specific Protection Goals.
- Providing context for running simulations with population/community models.

- Defining an objective basis for the evaluation of representativeness of higher-tier studies (e.g., field studies) and the extrapolation of findings from such experiments.

#### 2.04.P-Tu086 Metabolic-Based Identification of the Effects of Chemicals Using *Arabidopsis Thaliana*

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When chemical exposure occurs in the ecosystem, it carries the potential for profound environmental repercussions, particularly impacting terrestrial ecosystems. Notably, immobile plant species become particularly vulnerable in these scenarios, often serving as valuable indicators for assessing the extent of damage wrought by these chemical incidents. However, diagnosing and evaluating such damage currently lacks robust scientific methodologies, relying heavily on subjective visual assessment. In this context, the science of metabolomics emerges as a promising tool, enabling us to delve deeply into the physiological state of organisms in an environment marred by chemical exposure. Therefore, the focus of this study is to develop a methodology for assessing the implications of substances utilized in chemical accident preparedness. This is achieved by scrutinizing changes in the metabolites of plants exposed to chemical-laden environments through the lens of metabolomics, shedding light on the intricate repercussions of chemical accidents on the natural world. This study presented a methodological approach for understanding the effects of chemicals on plants through the application of metabolomics. For this research, the model plant, *Arabidopsis thaliana*, was used. *Arabidopsis* is a commonly used model plant in various metabolomics studies. Target chemical exposed to *Arabidopsis* at selected concentrations and general phenotypic differences like biomass in *Arabidopsis* were confirmed. Metabolites extraction process was performed from the exposed *Arabidopsis*, and non-targeted analysis was performed using Liquid and Gas Chromatography Ultra high resolution Quadrupole Time of Flight Mass Spectrometer System (LC-QTOF-MS). Then, statistical analysis was performed to distinguish the difference between the group that was not exposed to the chemical and the group that was exposed to the chemical. This study can help determine the effects of chemicals in plants using metabolomics.

#### 2.04.P-Tu087 How Does Biotransformation Vary Within and Across Species?

**Dave T. F. Kuo**, National Taiwan University, Taiwan

Biotransformation is important for accurate ecotoxicological assessment of chemicals as it influences bioaccumulation potential, toxicity, and persistence of chemicals in natural environments. Understanding how biotransformation differs across biological species or taxonomic grouping can thus provide important insights into interspecies difference and sensitivity of key ecotoxicological traits. This study aims at comparing and understanding how biotransformation rate constant,  $k_M$ , varies within and across different biological species, and how it may be affected by exposure conditions, environmental factors, and ecological traits. Using the early-time approximation developed earlier, a total of 241 tissue-specific unique experimental  $k_M$ 's were obtained for 61 unique species. Intraspecies uncertainty in  $k_M$  was typically within  $\pm 0.5$  log unit with additional variability explained by  $k_M$ 's dependence on exposure conditions and environmental parameters.  $k_M$  generally declined with higher exposure concentration regardless of exposure medium; the influence of temperature, inhibitor, and bioavailability modifiers were more ambiguous and of less important. Significant cross-species  $k_M$  differences have been observed among species within the larger biological groups of fish, bivalves, worms, and algae. Further analysis seems to suggest that omnivores may have the highest  $k_M$  than species in other feeding guilds. This dependence broadly agreed with the hypothesis that generalists with broad dietary choices may have evolved more mechanisms and diverse gut microflora to detoxify toxic moieties. This work demonstrates the possible integration of toxicokinetics into interspecies assessment framework and tools (e.g., species-sensitivity distribution) as well as the need for more comprehensive biological and ecological descriptors for building large-scale understanding of ecotoxicological data from through the lens of biology and ecology.

#### 2.04.P-Tu088 Changes in Pesticide Contamination With Age in Chicks of a Bird of Prey

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The massive use of synthetic inputs due to agricultural intensification is considered as a main responsible for farmland birds decline across Europe. While experimental studies investigated the toxicokinetics of some pesticides in model species, these do not reflect realistic rates in case of multiple pesticide contamination. Still, wild birds are exposed to pesticide cocktails and no study to our knowledge investigated the variation over time of their pesticide contamination *in natura*. We aimed at filling this gap using Montagu's harrier (*Circus pygargus*), a specialist (feeding on voles) bird of prey living in agroecosystems and nesting on the ground of cereal crop plots. Chicks are thus particularly interesting to study pesticide seasonal changes as they are born and raised in contact with the treated culture and fed with potentially contaminated prey. Consequently, their exposure to pesticides lasts all their growing period (four weeks) and may have important issues on their development. We used blood samples of Montagu's harrier chicks collected at the second and fourth nest visit, when they were ca. 15 days old and ca. 26 days old to investigate the changes in pesticides detected and their concentrations in-between. The detection and quantification of 104 molecules was realized on samples collected in 2018 (35 chicks) and 2019 (32 chicks). We detected in total 18 molecules in 2018 and 8 molecules in 2019. While no temporal pattern arises in 2019, in 2018 chicks sampled had on average two molecules less at the fourth visit than at the second visit. Similarly, the sum of pesticide concentrations was lower in older chicks. However, when regarding molecules' concentrations separately, only one individual had a significant reduction of concentrations between the two sampling dates. Overall, our results suggest that some molecules were metabolized and

detoxified notably through feather growth or stocked in the growing tissues, and/or that a natural depletion of pesticides in the environment occurred over time. These results question pesticides' exposure and detoxification routes in chicks. More investigations are thus needed to better understand the factors influencing pesticide's deposition and excretion within farmland birds. Nonetheless, our study gives insights on the temporal changes in pesticide contamination in blood matrices and highlights the complexity of comparison among birds sampled at different life-stages.

#### **2.04.P-Tu089 A Laboratory-Based Chronic Toxicity Model is Predictive of Nickel Effects on Benthic Invertebrates in the Field**

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Predicting effects of metals on stream invertebrate communities can be hindered by spatial and temporal variation in toxicity-modifying factors, a paucity of toxicity information for stream taxa (mainly insects), and variation in benthic invertebrate community structure related to habitat and factors other than the stressor of interest. We tackled these challenges by combining laboratory-based chronic toxicity data with field-based biological monitoring data to build a lab-to-field stressor-response relationship. A site-specific toxicity model for *Ceriodaphnia dubia* was used to translate nickel concentrations into predicted toxicity, and a quantile regression of field data from a wide range of sites was then used to describe the limiting effect of nickel toxicity on benthic invertebrate community metrics. The most sensitive metric was percent Ephemeroptera, with an EC<sub>10</sub> occurring at 22% effect to *C. dubia* reproduction. These findings support previous estimates of 20% as a critical effect size in laboratory test organisms that could translate into discernible effects on sensitive invertebrates in the field.

#### **2.04.P-Tu090 Ecotoxicological risk assessment for fish populations - Application to the early life stages of endangered migratory fish living in the Garonne catchment.**

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In the Garonne catchment (South West France), anadromous fish populations have severely decreased over the last decades. Among the potential causes of this decline, questions about water contamination have received limited attention. One of the existing methods to quantify potential toxic risk is called "Potentially Affected Fraction of species" (PAF). This approach consists of estimating the percentage of potentially affected species by comparing environmental contamination data and toxicity data. In our study, PAF approach was applied to quantify the potential toxic risk associated with chemical contamination of water for (1) fish in the Garonne and Dordogne rivers (main river scale) and (2) the development of early life stages in the spawning grounds (SG) of two emblematic species: allis shad and European sturgeon (SG scale). Environmental contamination data were provided for 21 sites from 2007 to 2022, and toxicity data were obtained specifically from freshwater toxicity tests on fish species. Then, for each year and site, the potential toxic risk associated with a single substance (ssPAF) and a mixture of substances (msPAF) was calculated and categorized as: high (> 5%), moderate (> 1% and < 5%) or low (< 1%). At both scales (main river scale and SG scale) and rivers (Garonne and Dordogne), the potential toxic pressure appeared to decrease over the period at most sites, with higher potential toxic risks between 2007 and 2012, although by 2022 few sites showed a low potential toxic risk. Moderate potential toxic risks (1% < msPAF < 5%) were more often observed, and high potential toxic risks (msPAF > 5%) were encountered more or almost as often as low potential toxic risks (msPAF < 1%). Lead (metals), copper (metals), zinc (metals), estrone (hygiene and care products), bisphenol A (other industrial pollutants) and iron (metals) were the higher contributors (ssPAF > 5%). Substances belonging to agrochemicals contributed at most moderately (1% < ssPAF < 5%). Acetochlor (agrochemicals) and 4-tert octylphenol (other industrial pollutants) generated moderate potential toxic risks (1% < ssPAF < 5%), specifically for the development of early life stages of fish in allis shad and European sturgeon SG. In conclusion, this study highlights the potential influence of water contamination on the decline, fate and restoration of anadromous fish populations in the Garonne catchment, focusing notably on the toxic effects on early life stages.

#### **2.04.P-Tu091 Exploring Embryonic Responses of a Migratory Fish to Spawning Grounds Water Quality in a Population Decline Context.**

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The allis shad (*Alosa alosa*) is an amphihaline migratory fish species, whose population numbers have drastically declined since the early 2000s and remain at a very low level in the Gironde catchment (France). Water quality at the spawning grounds, and its possible toxicity to the species embryos and larvae, is one hypothesis that has not yet been investigated, requiring continuous exposure of the embryos to a natural environment.

Early life stages of fish are sensitive to environmental conditions, notably water contamination, making them relevant for environmental monitoring applications, but also to water parameters. The *ex-situ* method allows a direct continuous exposure of organisms to river water, while controlling physico-chemical parameters (temperature, oxygen, photoperiod), known as confounding factors and to impact the results interpretation. Experiments were carried out on a spawning ground of allis shad in the Garonne river using *ex-situ* exposure method, in spring 2022 and 2023. Allis shad embryos were exposed to the



Garonne river water throughout their development from fertilization up to 2 days after hatching. Temperature was maintained at  $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and oxygen saturation values were higher than 80%, which is the ideal range for development.

Results showed a high mortality induced by the water of spawning grounds on both experimental years, and on three distinct batches of embryos. Specifically, embryonic mortality was significantly higher in individuals exposed to Garonne water compared to the control, with the probability of survival being less than 25% in the former, while survival in the control ranged between 50% and 90% depending on the batch. The most of embryonic mortality occurred after 30 hpf, or 24 hours of exposure which corresponds to organogenesis for this species.

Survival in controls demonstrates the interest of the *ex-situ* approach and water parameters regulation, allowing the exposure of early life stages in the natural environment. These results also show that the quality of the water has a significant impact on embryonic development, raising questions about the impact of water quality on allis shad population recruitment in the Garonne river.

#### **2.04.P-Tu092 Unravelling The Impact Of Climate Change On Aquatic Ecosystems: Insights From Historically Contaminated Soils In Estarreja, Portugal**

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The global efforts for the mitigation of climate change are formalized within the Paris Agreement. Multistakeholder engagement and local dimension are among the crucial elements for urgent climate adaptation. Our research project TERRA - Climate change impacts in Estarreja region focuses on local problems of Estarreja municipality, categorized as the most polluted municipality in Portugal, according to the World Health Organization. Human activities are leading to negative effects worldwide, not only in relation to the increasing amount of CO<sub>2</sub> released into the atmosphere but also to soil/water contamination, namely in the proximity to industrial chemical complexes (ICC), as the ICC in Estarreja, northern Portugal. Alterations in climate conditions may directly affect organisms by changing their behaviour, physiological state, *etc.* In addition, increased temperature and atmospheric carbon dioxide (CO<sub>2</sub>) levels may influence soil and water properties, affecting chemical bioavailability and, consequently, their toxicity to animals and humans.

The present study aimed to assess the effects of climate change on a polluted aquatic ecosystem using the historically contaminated area in Estarreja as a case study. For that, soil was collected from an agricultural area near the Estarreja ICC. Ecotoxicological bioassays with aqueous soil extracts, simulating run-off to surface waters, were conducted with aquatic species from different trophic levels: *Lemna minor*, *Daphnia magna* and *Danio rerio*. Tests were performed under two different climate scenarios: 1) a climate condition based on the IPCC predictions for 2100, with increased temperature and atmospheric CO<sub>2</sub> level (20-30°C ramp + 800 ppm atm. CO<sub>2</sub>); 2) and a standard climate condition recommended by OECD 221, 202 and 236 guidelines for each species. Results showed that *D. magna* survival decreased in Estarreja soil extract and was more pronounced in the increased temperature and CO<sub>2</sub> climate conditions compared to the control. The hatching success of *D. rerio* embryos and the growth rate of *L. minor* were negatively affected in exposures to the Estarreja soil extract in the increased temperature and CO<sub>2</sub> climate conditions (compared to the control medium). Within the scope of TERRA project, the present findings will be disseminated among the Estarreja population to increase awareness about soil and water contamination and the environmental impacts of climate change.

#### **2.04.P-Tu093 Development of an Ecotoxicological Bioassay Battery for the Integrated Assessment of Groundwater Systems**

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Germany obtains 70% of its drinking water from groundwater. Thus, Groundwaters of a very high overall quality status are of high importance for the consumers as much as for the ecosystem. Groundwater ecosystems are characterized by unique biodiversity with many rare, endemic and fossil species and are therefore, particularly worthy of protection. However, at many groundwater sites, there is a complex pollution situation due to the input of nitrates, pesticides, pharmaceuticals, and other industrial chemicals. A comprehensive assessment of the impact of this pollutant input on aquatic fauna and the ecosystem cannot be made with the common and in some cases mandatory chemical analysis of groundwater. Also, the ecotoxicological effects should be evaluated in addition to the chemical analysis of anthropogenic pollution and the status of the groundwater

fauna. The recording of this triad approach allows a comprehensive assessment of the anthropogenic impact on the groundwater body. It thus creates the basic knowledge required to protect groundwater ecosystems in the long term.

Such triad-based approaches have already been used very successfully for the holistic assessment of sediments and surface water. In the gwTriade project, a triad approach is to be developed for the first time, specifically for groundwater. Based on the gwTriade project, a broadly diversified ecotoxicological bioassay battery will be adapted to the particular physico-chemical challenges of groundwater (high sulphur, nitrate content) in order to investigate the ecotoxicological status. In addition, the stygophilic species *Eucyclops serrulatus* will be integrated into the bioassay battery. The aim here is to comparatively investigate how the simultaneous contamination of *Eucyclops serrulatus* and *Daphnia magna* (Crustacea) with groundwater contaminants and other stressors, such as heat and nitrate pollution, affect the sensitivity of the organisms. For this matter the development of *Eucyclops serrulatus* is systematically studied in order to design an ecotoxicological test method.

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#### **2.04.P-Tu094 Effects of Copper, Food Quality and Exposure History on *Chironomus riparius* Emergence: Insights From a Multigeneration Study**

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Emerging aquatic insects are important vectors for dietary nutrients such as long-chain polyunsaturated fatty acids (LC-PUFA) for riparian consumers. Aquatic contaminants may affect the transport of these nutrients by impairing development and emergence of aquatic insects. However, repeated or continuous contaminant exposure has hardly been studied against the background of aquatic-terrestrial linkages, but may facilitate the understanding of chronic effects on the quantitative and qualitative subsidy of terrestrial ecosystems. Therefore, we assessed the emergence success and timing of populations of the aquatic insect *Chironomus riparius* (Diptera: Chironomidae) when exposed to the essential metal copper (Cu), both before and after about six months of chronic exposure towards this stressor. The nutritional quality of emergent *C. riparius* were determined based on their FA profile. The quality of the basal food source provided to the larvae (Spirulina – low quality or TetraMin – high quality) was assessed as an additional factor triggering differences in emergence quantity and quality. Copper sediment contents predominately shaped the emergence success of *C. riparius* with reductions of about 30%, 60% and 90% at high Cu sediment contents (200-400 mg/kg dw). Furthermore, emergence was more strongly reduced when larvae were fed Spirulina compared to TetraMin (difference in effect size of 35% at 200 mg/kg dw). At 300 mg/kg dw, the number of emergent adults varied based on the exposure history of *C. riparius* (50% and 70% reduction in pre-exposed and control populations, respectively). Both high Cu sediment levels ( $\geq 200$  mg/kg dw) and lower basal food quality (i.e., Spirulina) delayed the median time until 50% emergence. The FA analysis is ongoing and will provide insights into the effects of chronic contaminant exposure on the transport of FA by emerging aquatic insects. Since LC-PUFA are mostly synthesized in the aquatic environment but are required for many physiological processes, riparian consumers appear to be particularly vulnerable to such changes.

#### **2.04.P-Tu095 Multi-generational exposure of *Daphnia magna* to pharmaceuticals: Effects on habitat selection behaviour and reproduction**

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Pharmaceutical products are a group of emerging environmental contaminants, whose presence in the aquatic environment poses a risk to organisms. In particular, these products are related to effects on lethality, reproduction, stress induction, neurotoxicity, development, as well as changes in the habitat selection processes by organisms. Although most studies focus on short exposures, these substances are increasingly present and are becoming part of aquatic ecosystems; therefore, it was aimed to assess how the multigenerational exposure of *Daphnia magna* to caffeine, ibuprofen, and fluoxetine could affect the reproductive capacity of organisms and their habitat selection behaviour. For this purpose, the crustacean *D. magna* was exposed to 10 µg/L of each pharmaceutical over three consecutive generations (F3). During this culture period, a daily neonates count was performed to determine the multigenerational effect of these contaminants on reproduction. In addition, habitat selection tests were performed in the HeMHAS (Heterogeneous Multi-Habitat Assay System) version #1, where organisms from F3 were provided to select areas with different contaminants and clean areas, under two scenarios: with and without food. As results, the presence of ibuprofen caused a delay of one week for the first brood, and a considerable reduction in the number of neonates per organism. On the contrary, *D. magna* populations cultured in caffeine showed a shorter time between generations and a greater number of neonates. In habitat selection tests, it was observed that most of the organisms that had been cultivated in clean water were in the compartment with caffeine, highlighting their higher attraction effect with respect to the clean water compartment. In the non-food experiments, all the populations pre-exposed to pharmaceuticals were distributed homogeneously in the system, with any particular preference. We showed that the presence of environmentally relevant concentrations of some pharmaceuticals could cause alterations on the habitat selection pattern and, therefore, on distribution patterns of this specie, as well as a significant effect on reproduction. As a consequence, at long-term, populations can be diminished at the local scale. These conclusions underline the importance of the study of pharmaceutical contamination

not only by understanding the short-term effects on biodiversity, but also long-term exposure, as well as the combination of multiple products.

#### **2.04.P-Tu096 Effects of antidepressants on *Daphnia magna*'s behavioural response**

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Selective serotonin reuptake inhibitors (SSRIs) are commonly used as antidepressants to treat mental disorders like depression. Specifically, its mode of action is to block serotonin reuptake and thus keep high serotonin levels. Currently the consumption of these pharmaceuticals is increasing and because of this, they are being detected in aquatic systems; however, there is not enough information on their effects on exposed organisms. The present study assessed the impact of two antidepressants, sertraline and citalopram, on the behavior of the Cladocera *Daphnia magna*. For this goal, the avoidance and colonization capacity of *D. magna* was studied at 24 h and 48 h, respectively, in the HeMHAS (Heterogeneous Multi-Habitat Assay System) version #3. Exposure was at environmentally relevant concentrations: 0, 0.1, 0.5 and 1 µg/L. We observed that there was no avoidance in *D. magna* to these pharmaceuticals. The response to sertraline was concentration-dependent ( $F_{3,12}$ : 6.139;  $p < 0.05$ ), with a significantly higher percentage of organisms in the compartments of 1 µg/L, compared with control. Citalopram caused also a concentration-dependent attraction ( $F_{3,12}$ : 4.396;  $p < 0.05$ ), although the distribution of organisms was more homogeneous between compartments. As for the colonization tests, organisms lasted shorter time to reach the highest sertraline concentration. The organisms only needed 90 min to reach the last compartment, instead of 210 min as in the case of control. This response was not so marked in presence of citalopram, as organisms needed more time to reach the compartment with 1 µg/L of contaminant. However, after 24 h the percentage of organisms in that compartment was two-fold of organisms found in the highest concentration of sertraline. These results suggest the influence of this type of pharmaceuticals on the free distribution of organisms, causing a possible attraction, which can pose a risk in the case of causing an imbalance in the way to detect other contaminants and other environmental stressors.

#### **2.04.P-Tu097 Can E-wastes Modify Behavioural Responses of the Crustacean *Atyaephyra demarestii*?**

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Electrical and electronic products are part of our daily lives. This situation has experienced a remarkable growth in the last two decades with the increasing digitalization of society. The generation of waste from technological products, known as e-waste, represents a global environmental problem of great concern, as many of these products contain metallic elements that are potentially toxic and can pose a health problem to ecosystems and humans. Electrical and electronic devices generally have a printed circuit board on which a number of circuits are printed with metals. Some of them are rare earth elements such as erbium (Er), cerium (Ce) and neodymium (Nd). However, the case of lithium (Li) deserves a special mention, as its use has increased considerably for its application in batteries of electric and hybrid vehicles. Understanding how the release of these elements can affect aquatic species and ecosystem biodiversity is a challenge. In this work, we have analyzed how the occurrence of these elements in individual form and as binary mixtures can trigger the avoidance behavior of the crustacean *Atyaephyra demarestii*. The experimental setup has been carried out using the HeMHAS (Heterogeneous Multi-Habitat Assay System) for 24 and 48 h, which has allowed it to calculate the relationship between a gradient of exposure and avoidance behavior for individual and mixture elements. In this study, it is shown how E-wastes affect the habitat selection behaviour of shrimps and their ability to interact with the surrounding environment.

#### **2.04.P-Tu098 Contamination and habitat fragmentation as drivers of population distribution of stressed landscapes**

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Changes in the connectivity configuration of landscapes (i.e., habitat fragmentation) and contamination may have long lasting effects on biodiversity, such as the local extinction of species and habitat use. The aim of this study was to evaluate the interaction effects of habitat fragmentation and contamination on the habitat selection by zebrafish (*Danio rerio*), and their ability to explore landscapes with different connectivity configurations and a heterogeneous spatial arrangement of contamination. It was hypothesized that both contamination and habitat fragmentation reduce the probability of organisms finding resources (i.e., food) by increasing the time spent foraging in fragmented landscapes and by avoiding contaminated areas. For this purpose, the organisms were exposed to three scenarios of contamination (0, 0.5 and 25 µg/L of copper) and physical fragmentation (zero, low and high), using the Heterogeneous Multi-Habitat Assay System (HeMHAS). Fish were stimulated to forage by adding food in the last compartment at the end of the system. All experimental trials were videotaped, and the time spent to find food and within each specific compartment were recorded. Generalized Bayesian linear models were used to analyze the data and evidence ratios (ER) were used to test the hypotheses. Strong evidence suggests that the spatial distribution of organisms was hindered as consequence of both high levels of fragmentation and contamination (ER > 1000 and ER = 122 respectively). However, no significant effects were observed in the probability of organisms finding food and the time spent in the low fragmented and contaminated scenarios (ER = 1.56 and ER = 0.76 respectively). Overall, organisms avoided highly contaminated areas by spending less time in compartments with high concentrations of copper and more time in

fragmented compartments. These results indicate that the probability of finding resources was reduced by the effect of contamination, and the time spent in the system foraging and within each compartment was linked to the combination of the contamination and fragmentation. As a conclusion, the contamination and fragmentation had a noteworthy synergistic effect over the spatial distribution of fish, affecting the probability of reaching food and the time spent to achieve it. Also, the HeMHAS demonstrated its suitability to assess the role of the contamination and fragmentation stressors in the spatial distribution and habitat selection response.

#### **2.04.P-Tu099 Shy vs. bold: Testing if personality traits influence zebrafish propensity to colonize polluted environments**

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Individual differences in fish behavior, also known as personality traits, contribute to animal fitness by influencing social interactions, foraging success, and predator avoidance. Personality could also affect decision making in unpredictable environments, such as polluted habitats. Bolder fish, for example, tend to colonize new environments faster, potentially increasing their risk of exposure to contaminants. Although research has begun to test how exposure to contaminants affects fish behavior and personality, to what extent personality itself influences the exposure of fish to contaminants via their propensity to enter and stay in contaminated habitats is still unclear. Here, we assessed how fish personality traits (shy and bold) affected their habitat colonization and habitat choice in a gradient of pollution using zebrafish as a model species. We used two standard tests for boldness in fish: novel environment and refuge seeking. We profiled each individual as bold or shy, based on their swimming behavior and positions in the tank. We then tested habitat choice using the Heterogeneous Multi-Habitat Assay System (HeMHAS), a multicompartiment arena where the organisms can move freely along a chemical gradient. We tested each fish along a linear gradient of two chemicals: fluoxetine and copper (0.1-100 µg/L), as well as a clean water control. We selected fluoxetine as it is a contaminant of emerging concern that is known to attract fish. Copper was used as a positive control, for its known aversion effects on zebrafish. The personality test we used successfully discriminated between the two phenotypes (bold-shy). For habitat choice, we expect that both bold and shy fish show an avoidance towards copper and attraction towards fluoxetine as has been reported previously for this species. However, we expect to see differences between phenotypes in the colonization time to polluted compartments, and residence time in each compartment. We predict that bolder fish will colonize the compartments with higher concentrations of fluoxetine faster than shy fish, and only bold fish will colonize compartments with high concentrations of copper. As concerns regarding emerging pollutants like pharmaceuticals grow, understanding how personality can shape the exposure of animals to environmental stressors becomes crucial. Our study contributes to this evolving field looking at the intersection of animal personality and environmental pollution.

#### **2.04.P-Tu100 Ecotoxicological Bioassay of Commercial Sunscreens on *Phaeodactylum Tricornutum***

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The European coast receives millions of tourists each year and significant amounts of commercial sunscreen (SSn) compounds are being released into aquatic environment. Until now there is a significant gap in the information concerning the toxicity of SSn on marine microalgae. Thus, the effect of SSn at different concentrations (0, 5, 10, 25, 50, 100 and 200 mg.L<sup>-1</sup>) were assayed on the growth of the most common species of marine microalgae used in ecotoxicology: *Phaeodactylum Tricornutum*. Culture were exposed under controlled conditions in a climatic chamber with continuous agitation at 20 °C, and salinity of 36 PSU in a 96 h study using flow cytometry analysis (FCM). Reactive oxygen species (ROS) production were monitored by 2',7'-dichlorofluorescein (DCFH) using FCM. Chlorophyll was measured by auto-fluorescence. The Results indicated that *P. Tricornutum* sp. was significantly more sensitive at the highest concentration 200 mg.L<sup>-1</sup> of SSn used compared to control. As well as, algal growth revealed that in the absence of sunscreens, *P. Tricornutum* grown similarly to two lowest concentrations (5, 10 mg.L<sup>-1</sup>). Chlorophyll *a* fluorescence showed a significant decrease ( $p < 0.05$ ) in *P. Tricornutum* under three highest concentrations (50, 100 and 200 mg.L<sup>-1</sup>). ROS levels were triggered under 100 and 200 mg.L<sup>-1</sup>, by 66 and 63% respectively; which was higher than all the others treatments. Our study resulted in data concerning the potential impacts caused by possible future drastic effect of commercial Ssn in aquatic habitats.

#### **2.04.P-Tu101 Effects of microalgae based biostimulants on water and soil condition and species**

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The growing global population and rising food consumption have inevitably raised the demand for agricultural food products. In addition, growing environmental awareness prompts questions about agricultural practices and how they affect biodiversity and ecosystem function. The effects of these production processes on environmental quality and climate change are of critical importance. It is important to achieve a balance between efficient food production and sustainable agricultural methods. Developing such a scenario requires a multifaceted approach which includes optimized nutrient input and an increased crop tolerance to environmental stress. To this end, innovative technologies based on bioresources include the use of microalgal biostimulants to improve crop yield while lowering the environmental footprint of agriculture. Today, there is a lack of scientific data on microalgae used as biostimulants and its environmental impact, which hinders the advancement and

establishment of this technology. Specifically, there is a lack of knowledge regarding the underlying mechanism of action and the effectiveness of microalgal biostimulants in improving agronomic efficiency. In this study we examine the potential ecotoxicological impact of microalgal biostimulants on non-target aquatic and terrestrial organisms. We performed toxicity tests to document and understand the potential ecotoxicological impact of microalgal biostimulants (*Spirulina platensis*, *Chlorella vulgaris*, *Nannochloropsis gaditana* and *Dunaliella salina*) on a range of representative organisms for both terrestrial and aquatic ecosystems. The tests were performed at concentrations of 1g/L, 0.5g/L, 0.25g/L, 0.1g/L, 0.05g/L, 0.01 g/L with model organisms *Daphnia magna* (water flea) 48 hours mortality/immobilization test and 21 days reproduction test, *Eisenia fetida/andrei* (earthworm) 21 days mortality test, *Branchionus calyciflorus* (rotifer) 24, 48 hours mortality/ immobilization test- and *Spirodela polyrhiza* (duckweed) 72 hours toxicity test. At the highest concentrations (1g/L and 0.5g/L) of *Spirulina platensis* and *Chlorella vulgaris* there was high mortality in *Daphnia magna* 48 hours test which is indicative of an ecotoxicological impact on the environment. So far optimal biostimulant concentrations seem to be reached at concentrations < 0.1 g/l and chronic assessments are being conducted at this and lower concentrations.

#### **2.04.P-Tu102 Evaluations of aqueous toxicity and cytotoxicity of Ziram**

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The grass carp, is one of four invasive carp species established in North America. The other invasive carp are bighead carp, black carp, and silver carp. Grass Carp have been recorded in 45 states in the United States, with spawning populations in the Great Lakes and the Missouri and Mississippi Rivers. Therefore, fisheries managers are interested in identifying new chemicals that can be used for the selective control of invasive carp. These new chemicals should show some species-specificity, be palatable to target species, demonstrate low persistence in aquatic systems, and have minimal adverse effects on non-target species. One such chemical is Ziram, a dimethyl-dithiocarbamate containing zinc (log  $K_{ow}$ :1.23) is currently registered as an agricultural fungicide that may have promise to control undesirable fish, like the Grass Carp. Few studies have measured the toxicity of Ziram to fish or freshwater invertebrates, and many of these studies were based on nominal concentrations. The objectives of this study were to evaluate the potential acute toxicity of Ziram in an aqueous solution to a suite of non-target aquatic fish and invertebrates and determine the *in vitro* cytotoxicity (OECD 249) of Ziram in Grass Carp, Lake Sturgeon, and Rainbow Trout gill cell lines. A total of six species, three fish and three invertebrates, were exposed to a series of aqueous Ziram concentrations for 96 hours and experimental concentrations were analytically verified in exposure media for total Zn concentrations. In the invertebrate bioassays, 96-h LC<sub>50</sub> values (nominal concentrations) ranged from 0.14 mg/L to greater than 2.0 mg/L, with amphipods being the most sensitive species. In the fish studies, 96-h LC<sub>50</sub> (nominal concentrations) values ranged from 5.4 µg/L to 354 µg/L, with Lake Sturgeon being the most sensitive species. Similarly, 24-h fish gill cytotoxicity results indicated Lake Sturgeon were the most sensitive species to Ziram. We compared cytotoxicity EC<sub>50</sub> values to toxicity values from *in vivo* exposures. Preliminary results indicate relative agreement between cytotoxicity and *in vivo* toxicity values for Rainbow Trout and Grass Carp, and the ratio of *in vitro*: *in vivo* 24-hour toxicity values is less than 2-fold. Toxicity data will be used to determine whether Ziram toxicity is species-specific and sufficient to develop a prospective Ziram formulation for effective invasive carp control.

#### **2.04.P-Tu103 Feeding activity of gammarids as ecotoxicological endpoint for acute fungicide stress in freshwater mesocosms**

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Biodiversity as well as ecosystem functioning of aquatic fungi (AF) can be significantly influenced by fungicides. Furthermore, shredders feeding on contaminated leaves may also be affected directly by fungicide exposure (i.e. *via* water phase or fungicides adsorbed onto leaves) or indirectly *via* changes in food quality due to effects on the leaf-associated microbial community. As part of a comprehensive mesocosm study investigating effects of the fungicide Tebuconazole (TBZ; product Folicur®) on fungal biodiversity and functioning in freshwater mesocosms, an *in-situ* experiment using *Gammarus roeseli* feeding on alder leaves was performed. The aim of the *in-situ* experiment was to investigate if the feeding activity of *G. roeseli* can be used as sublethal endpoint in mesocosm studies to evaluate pesticide stress. Eight near-naturally established freshwater mesocosms were dosed once with six different concentrations of TBZ (5-5000 µg/L). The feeding activity of *G. roeseli* was measured after two and six days of TBZ exposure in the contaminated mesocosms under different water temperatures, using leaves previously colonized in control water for three weeks (experiment 1). In experiment 2, leaves were conditioned for 3 weeks in mesocosm water treated with TBZ concentrations and were then used for the feeding experiment with exposure of *G. roeseli* in control and treatment mesocosms (test durations also two and six days). Each experiment consisted of four cages per mesocosm, each containing three *G. roeseli* and ten leaf disks. After the experiment, the remaining leaf disks were photo-documented and weighed to measure leaf loss. The feeding activity of *G. roeseli* was clearly inhibited with TBZ exposure using uncontaminated leaves and showed a clear concentration response relationship. Lowest EC<sub>50</sub> was 264 µg/L TBZ (2d, 19°C, experiment 1). In experiment 2, feeding activity of *G. roeseli* was reduced by ± 20 %, in which gammarids fed contaminated leaves for two days in control water. However, inhibition of feeding activity of gammarids was negligible in the same experiment when exposed for six days except for the highest concentration. Possibly, hunger effects as well leaching of TBZ from leaves into the uncontaminated medium (analytically measured) may have led to a recovery effect. Inclusion of *in-situ* feeding experiments in mesocosm studies seems to be a promising tool to investigate short-term acute sublethal effects on gammarids.

#### **2.04.P-Tu104 Using Individual Biomarkers to Characterize the Effects of Pesticide Mixture in *Gammarus fossarum* Under Mesocosm Conditions**

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Pesticide contamination of continental freshwater bodies is now widespread. In agricultural landscapes, constructed wetlands (CW) are set up to intercept and trap pesticides. Pesticides then undergo purification processes linked to physical (sorption) and biological (degradation) processes through interaction with plants and the activity of microorganisms. However, these pesticide-trapping wetlands could act as ecological traps, i.e., habitats that are potentially attractive to species but reduce the fitness of organisms. In addition, little is known about pesticide cocktail effects in general, and in these environments in particular. The aim is to identify the potential effects of a pesticide cocktail in a CW with a model species at the individual scale. Our study site is a French CW of 3,500 m<sup>2</sup> treating contaminated water from a 355 ha agricultural watershed planted with cereal, representative of intensive agriculture. The model species is the crustacean amphipod *Gammarus fossarum*. In six "in situ" 300L mesocosms (2m × 0.5m × 0.3m), located in the center of the CW, but isolated from the surrounding water, four cages containing 20 gammarids and alder leaves (*Alnus spp.*) were immersed for one week, and the experiment was repeated four times with different groups of gammarids each time (i.e. 4 weeks of independent exposure × 6 mesocosms × 4 cages × 20 gammarids). We used a cocktail of nine molecules, herbicides and fungicides, representative of the main pesticides found in the watershed and in the CW in the last ten years. This cocktail was injected once, in four of the six mesocosms, at the beginning of the four consecutive weeks of exposure from March 25 to April 22, 2021. We monitored four individual biomarkers: survival rate, locomotion rate, amplexus rate (reproduction) and feeding rate. The main results are: (i) survival rate is not significantly different between pesticide-exposed gammarids and unexposed gammarids, (ii) increasing pesticide exposure increases locomotion rate. The latter result may reflect pesticide avoidance behavior in exposed gammarids. Pesticide avoidance by certain invertebrates could have implications for the structure and ecological functioning of CWs under agricultural chemical pressure.

#### **2.04.P-Tu105 Unravelling the Molecular Mechanisms of Fish Salinity Adaptation in the Face of Severe Osmotic Stress: A Comparative Multi-Tissue Transcriptomic Study in the Llobregat River, Barcelona, Spain**

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Freshwater salinization, driven by activities such as potash mining, is a global environmental concern threatening freshwater biodiversity, particularly fish populations. This study focuses on the intricate molecular mechanisms guiding fish adaptation to rising salinity levels in the urbanized Llobregat River, Barcelona. Employing high-throughput mRNA sequencing, we explored tissue-specific responses in the brain, gills, and liver of the invasive minnow (*Phoxinus septimaniae* × *P. dragarum*) at two distinct stations—a heavily impacted salt-polluted station near the "Potasses del Llobregat" potash mine and a reference station. Salinization significantly influenced global gene expression profiles, with the brain displaying the highest number of differentially expressed genes, highlighting its unique sensitivity to salinity fluctuations. Pathway analyses revealed enrichment of ion transport and osmoregulation pathways across all tissues, alongside tissue-specific pathways related to stress, reproduction, growth, immune responses, methylation, and neurological development in the context of salinization. Rigorous validation of RNA-seq data through quantitative PCR (qPCR) underscored the robustness and consistency of our findings. This investigation unveils intricate molecular mechanisms steering salinity adaptation in non-native minnows facing diverse environmental stressors. Our study provides crucial insights into the adaptive strategies of aquatic organisms grappling with freshwater salinization. Prioritizing efforts to mitigate potash mining pollution is recommended to counteract the physiological adaptive advantage conferred to invasive species over their native counterparts. This comprehensive analysis sheds light on the underlying genetic and physiological mechanisms governing fish adaptation in salinity-stressed environments, offering essential knowledge for the conservation and management of freshwater ecosystems facing escalating salinization pressures.

#### **2.04.P-Tu106 Weight-of-evidence approach for watershed-scale ecological risk assessment near a zinc smelter in the Nakdong River and Andong Lake, Korea**

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The upstream of Nakdong River and Andong Lake is contaminated by heavy metals such as Cd, Zn, As, and Pb. Although the origin of the contamination is unequivocal, it is suspected that the heavy metals have been leached from zinc smelter. Traditional ecosystem assessment, focusing on specific chemicals, faces doubts regarding its effectiveness in translating laboratory findings to real ecological impacts using lab ecotoxicity studies and field measurements for risk evaluation. In this study, we conducted a weight-of-evidence (WoE) approach to better understand the ecological impacts of heavy metal contamination in the upstream of Nakdong River and Andong Lake. The WoE analysis integrated evidence from a variety of sources, including field monitoring of metal concentrations in sediments, sediment elutriate and surface water, toxicity tests on sediments and sediment elutriate, and surveys of the biota.

Analysis of heavy metal concentrations in sediments and surface water revealed elevated hazard quotients(HQ) for As and Cd in sediments and Zn and Cd in surface water. The concentrations of Zn and Cd were notably elevated in the surface water downstream of the smelter, and the hazard decreased after 70 km. Toxicity tests using *H. azteca* on sediment site samples

showed that 57% of the samples were toxic. Logistic regression analysis of the sediment toxicity and mean Probable Effect Levels quotients(mPELq) showed a significant correlation, and Cd and As were identified as the primary contributors to the observed toxicity. In contrast, sediment elutriate toxicity tests showed that 18% of the samples were toxic to *D. magna*, and Zn was identified as the primary contributor to the observed toxicity. Benthic macroinvertebrate surveys were used to assess Benthic Macroinvertebrate Index(BMI) and Species At Risk(SPEAR) of metals at 12 sampling sites in the upstream of Nakdong River. Bioavailability toxic unit(TU) derived using the Biotic Ligand Model(BLM) and Hardness correction showed a significant correlation with SPEAR<sub>metal</sub>. Cd and Zn were identified as the primary contributors to the observed toxicity.

These findings suggest that the Zn and Cd contamination is having a significant impact on the ecological health of the upstream of the Nakdong River and Andong Lake. The results of this study provide important information for environmental management and remediation efforts in the area.

#### **2.04.P-Tu107 Watershed-Level Characterization of Chloride Exposure and Associated Risks: A Case Study in One of the World's Largest Watersheds**

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The concentrations of salts in freshwater systems have been increasing globally, resulting in concern of adverse and sustained ecological impacts. With numerous potential pathways for dissolved salts to enter surface waters (e.g., road de-icing, agriculture, wastewater effluents, and resource extraction), there is a need for high quality exposure data to inform risk assessors when making regulatory decisions for freshwater protection. To assess the ecological risk associated with freshwater salinization in Canadian surface waters, we used the Lake Winnipeg watershed as a case study. This is among the largest watersheds in the world, with an area of ~1 million km<sup>2</sup>. We were interested in characterizing the current state of salinity and salinization, as evaluated through the lens of a community-based monitoring (CBM) program. CBM volunteers gathered samples over three field seasons (2020-2022), from a total of 119 sites across Manitoba, with approximately 20 samples per site collected per year. The collected water samples were analyzed for chloride ion concentrations and each sampling site was categorized by their dominant land-use (e.g., urban, rural municipality, agriculture, pasture, lake outlet, and forest) to determine the driving sources, as well as temporal trends of salinization in the watershed. During the three field seasons, multiple sites across differing dominant land-uses exceeded the Long-Term Canadian Water Quality Guideline (CWQG) for the Chloride Ion (120 mg Cl<sup>-</sup>/L) based on time-weighted averages for each site, with 12.3%, 17.3%, and 3.6% exceedance rates in 2020, 2021, and 2022, respectively. There was a low acute risk of exceedance across sites; however, two urban sites exceeded the Short-Term CWQG (640 mg Cl<sup>-</sup>/L) multiple times in 2020 and 2021. The sites that experienced the greatest salinity levels were in rural municipalities within the Red River Valley and near the city of Winnipeg, Manitoba, suggesting that anthropogenic factors are likely driving exposure in these regions. Overall, this project will provide data to inform risk assessment, as well as identify a possible driver of ecological change in freshwater systems, while also enhancing public participation in their protection.

#### **2.04.P-Tu108 A Critical Review of Sodium Chloride Freshwater Toxicity Data for Generating Species Sensitivity Distributions**

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Rising ion concentrations from salts could pose a direct and indirect risk to freshwater organisms and irreversible impairment on the structure and function of food webs. Additionally, concerns about data quality, reporting and fit of toxicity data for use in risk assessment have been raised, which may lead to ecological impacts if used to generate insufficiently protective guidelines. Thus, high quality toxicity data are needed to inform risk assessors when making regulatory decisions regarding freshwater protection for chloride. To address the concerns surrounding the reliability and relevance of ecotoxicological literature, we conducted a critical review with the overall objective to assess the current state of laboratory toxicological data as it relates to chloride ions associated with freshwater salinization. Specifically, we sought to 1) evaluate the strength of methods of aquatic toxicity studies for sodium chloride on freshwater organisms, as well as their utility for ecological risk assessment via objective scoring rubrics; 2) use studies deemed suitable for risk assessment to create Species Sensitivity Distributions (SSDs), where it is possible to estimate water quality protection thresholds; and 3) identify major knowledge gaps and make recommendations to address current and future toxicity data needs related to freshwater salinization. A total of 313 papers were reviewed. Of these, 98 papers (with 358 data points from unique bioassays) met the inclusion criteria (as of November 2023) and were scored based on their utility for risk assessment. In general, studies described the test substance and test organism well, but there was room for improvement when reporting response results and test conditions. SSDs were created using the strongest chloride toxicity data to contrast with existing water quality guidelines and criteria. The hazardous concentration estimate for the 5<sup>th</sup> percentile of the distribution (HC<sub>5</sub>) values were determined to be 380 mgCl<sup>-</sup>/L from 56 species and 40 mgCl<sup>-</sup>/L from 52 species for the acute and chronic SSDs, respectively. In contrast, the Short-Term and Long-Term Canadian Water Quality Guidelines for the Chloride Ion are 640 mgCl<sup>-</sup>/L and 120 mgCl<sup>-</sup>/L, respectively. Therefore, this research provides data that is useful for ecological risk assessment and freshwater protection, as well as guiding future research by identifying specific species and exposure scenarios that might be lacking reliable and ecologically relevant data.

#### **2.04.P-Tu109 Exploring Trait-Based Vulnerability in Korean Freshwater Systems to Enhance Understanding of Heavy Metal Impact on Biodiversity**

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Recently, efforts have been made to integrate the distinct practices of biomonitoring and chemical monitoring, traditionally conducted separately, in assessing environmental status. Some researchers used the trait-based approach with biological and ecological information to examine how organisms respond to chemicals. For example, ecological vulnerability, encompassing exposure, recovery potential, and sensitivity to chemicals, relies on multiple trait indicators, enabling the prediction and assessment of organisms' overall states. Understanding the intricate interplay between organisms and chemicals in their environment is promising, especially in the context of preserving biodiversity. This study delved into trait-based vulnerability regarding four heavy metals (Cu, Cd, Pb, and Zn) in Korean freshwater systems. Traits encompassing various modalities served as vulnerability indicators grouped into exposure, recovery, and sensitivity categories. In addition, toxicity indicators for the heavy metals were incorporated to derive the vulnerability scores for target organisms. As a result, the vulnerability scores among different taxonomic groups showed significant differences. Fish displayed lower vulnerability to Cd and Cu than other groups, while Crustaceans, gastropods, and bivalves shifted in their vulnerability rankings due to toxicity indicators. Including the toxicity category markedly altered vulnerability scores for bivalves, especially for Pb and Zn. Such findings offer valuable insight into biodiversity preservation policies. Understanding how different species respond to specific chemicals emphasizes the necessity for customized conservation plans. Policymakers can implement focused actions, like stricter regulations or protective measures, for vulnerable species to chemical pollution. Incorporating these scientific insights into environmental policies will support safeguarding at-risk species, contributing to diverse ecosystem preservation.

#### **2.04.P-Tu110 Is Agricultural Pesticide Pressure of Prime Relevance for the Composition of Macroinvertebrate Communities in Small Agricultural Streams?**

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The German Federal Environment Agency commissioned a surface water monitoring campaign (2018-2019) to assess the chemical and biological condition of small agricultural watercourses and verify the effectiveness of risk assessment and management in the pesticide approval process. For more than 100 stream sites, water samples were analysed for pesticide active substances, pharmaceuticals and other urban contaminants, and aquatic macroinvertebrates were sampled to determine the community composition. The campaign provides a large monitoring dataset and can help to understand the chemical and ecological situation of small agricultural streams. The final campaign report concluded that the detected concentrations of pesticide active substances are the dominant stressor for vulnerable insects and the macroinvertebrate community. This conclusion is based on a strong correlation between toxic pressure attributed to pesticide active substances and SPEARpesticides, a biological index based on the proportion of the abundance of macroinvertebrate species classified as vulnerable to pesticide pressure. It is the nature of any biological index to correlate with the stressor it was optimized for and the report shows this e.g. for the saprobic index, explained primarily by deficient oxygen conditions and the EPT index explained by habitat factors hydro-morphology, streambed structure and flow. The present study questions whether the relevance of individual stressors can be assessed by means of specific biological indices and aims to shed light on the influence of abiotic factors on the composition of macroinvertebrate communities. The publicly available raw data from the campaign were analysed using ordination methods that, unlike indices, do not require pre-weighting species in terms of their assumed vulnerability to a specific stressor. Instead, species occurrence and abundance are used to group the stream sites and to relate them to various environmental factors. The results showed that that environmental factors such as water quality and quality of habitat structure were more relevant than pesticide pressure. In conclusion, SPEARpesticides can indicate the ecological status of small streams but does not necessarily clarify the multiple environmental factors impacting the status. Without this clarification, the role of pesticide pressure resulting from agriculture cannot be understood well enough to conclude on the pesticide approval process.

#### **2.04.P-Tu111 Regulatory and practical considerations on the implementation of a Mixture Allocation Factor in REACH**

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The exposure to various chemicals originating from diverse anthropogenic sources and through different pathways poses potential combined risks that current European chemical legislations do not adequately address. In its Chemical Strategy for Sustainability, the European Commission (EC) proposed introducing a mixture allocation factor (MAF) into REACH Annex I. This is to address risks from unintentional mixtures by introducing a “new” ceiling for the accepted risk during the chemical safety assessment of single chemicals. Focusing on environmental risk assessments, we critically discuss the MAF as most pragmatic and immediately implementable regulatory measure to account for risks arising from unintentional mixtures. We provide a 12-point action plan with practical recommendations and aspects to be considered for the introduction of a MAF in REACH. To enhance compliance and acceptance, we emphasize the importance of clarity and transparency with respect to the rationale, scope and selection of methods and data basis to define an appropriate magnitude for the MAF. Although it was announced by the EC, a public disclosure on details of the proposal and an analysis of socioeconomic impacts, the effectivity and benefits of introducing a MAF and a comparison to more specific regulatory measures are pending. We argue for the



application of the MAF as a generic risk management tool applicable to all REACH chemicals, without derogations and irrespective of tonnage, use, or environmental compartment. The MAF size should be based on the available different lines of evidence and set at a sufficiently high value to address uncertainties. The effectiveness of the MAF hinges on the implementation, application and enforcement of the MAF and the corresponding risk mitigation measures in order to effectively reduce environmental exposure levels of particularly those chemicals contributing to unacceptable risks for the environment. Still, assessing unintentional chemical mixtures must extend across various relevant chemical regulations in order to approach the real-life exposure situation and ensure harmonization. This is crucial not only for achieving the European Zero Pollution Ambition outlined in the European Green Deal but also for aligning with the global Sustainable Development Goals.

#### **2.04.P-Tu112 Variability of responses to multiple chemical stressors in invasive mosquitofish**

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Rapid adaptation to pollution in wildlife is still debated. For instance, recent studies document fish adaptation to dioxin like pollutants, but a limited number of empirical studies in realistic environmental conditions are available so far. In addition, chemical pollution in anthropized freshwater streams is frequently characterized by a cocktail of chemicals including several historical and emerging compounds at sub-lethal concentrations, which could complicate the evolutionary outcome of pollution exposure in the wild. In this study, we evaluated the phenotypic divergence of wild populations of invasive mosquitofish (*Gambusia holbrooki*) exposed to gradients of multiple chemical stressors in French rivers. In addition, we tested the inter-population divergence in tolerance to an experimental exposure to a cocktail of non-persistent pollutants to test for potential local adaptation to pollution. We hypothesized that increased tolerance to pollutants in fish living in polluted streams would be associated with: (i) no or moderate detrimental effects of chemical stressors on fish health, due to increased detoxication metabolism and/or higher non-enzymatic antioxidant defenses and (ii) a higher tolerance to an experimental exposure to a realistic cocktail of pollutants. First, we performed an extensive field study in 12 populations and compared the phenotype of F0 fish living along gradients of chronic pollution. Second, we experimentally tested the survival of their F1 offspring to an experimental cocktail of non-persistent pollutants (pesticides-pharmaceuticals). Results show that F0 fish from highly polluted sites had an increase biotransformation activity and a decrease non-enzymatic antioxidant capacity associated with limited oxidative damages. In addition, their F1 offspring had a higher survival when experimentally exposed to a realistic cocktail of pollutants, revealing a higher tolerance of juveniles coming from the most exposed populations. Further studies are now necessary to investigate the underlying genetic and epigenetic mechanisms explaining this phenotypic divergence.

#### **2.04.PC Bridging the Gap between Exposure, Ecotoxicology and Ecology – Identifying and Regulating the Impact of Chemical Pollution on Biodiversity**

##### **2.05.A Marine Ecotoxicology: Impacts and Possible Solutions, From Coastal to Deep-Sea Ecosystems**

**2.05.A.T-01 The Impact of 17 $\alpha$ -Ethinylestradiol (EE2) on Behaviour and Morphology in an Australian Marine Fish**  
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Due to the growing use of medication globally, the number of pharmaceutical pollutants in the environment has risen sharply. One pharmaceutical of particular concern is 17 $\alpha$ -ethinylestradiol (EE2), a synthetic estrogen used as the primary ingredient in the human contraceptive pill. In the environment, EE2 can alter morphology and physiology of exposed wildlife and increase mortality. However, the majority of EE2 studies have focused on freshwater species, despite the fact that EE2 is prevalent in marine systems. Accordingly, this study aimed to examine the impact of a range of environmentally relevant concentrations of EE2 on the behaviour and morphology of an Australian native marine fish—the crested weedfish (*Cristiceps australis*). Weedfish were exposed to one of three treatment groups using a static renewal system—an unexposed control (marine water; 0 ng/L), low-dose EE2 (measured concentration: 33 ng/L), or high-dose EE2 (measured concentration: 70 ng/L)—for 14 days. Following the exposure period, individuals were subject to a refuge-use assay to investigate the impact of EE2 on boldness and anxiety. Using IDtrackerAI, an automated AI tool for quantitative analysis of animal behaviour, specific behaviours including latency to enter the refuge, time spent out of refuge and overall activity were scored. Moreover, the influence of exposure treatment on morphological characteristics, such as body condition, length and weight, were also analysed as they are closely correlated with boldness and anxiety. Preliminary analyses show that EE2 has an impact on both behaviour and morphology in weedfish, in which fish exposed to the high treatment of EE2 had an increased body condition relative to the low treatment. We also revealed that fish in the high EE2 treatment were less bold and slower to enter the refuge than unexposed fish. Our findings will have repercussions for understanding consequences for population and species persistence in a polluted world.

##### **2.05.A.T-02 Bioplastics as Modulators of the Marine Bacterial Community in a Mesocosm Experiment**

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Biodegradable bio-based plastics (bioplastics) are promising materials in the substitution of non-degradable fuel-based plastics, but they are not exempt from the incorporation of additives that can leachate into seawater. Due to the recent transition to biomaterials, their effects on the marine environment and ecosystems are not fully understood. A closer look into their influence on marine microbial communities, base of the marine food web and essential regulators of marine biogeochemical cycles, provides first insights into bioplastics' effects in the oceans. In this work we studied the bacterial communities inhabiting coastal surface seawater after 2 months of exposure to different bioplastic materials in their raw and commercial format, in a mesocosm experiment simulating natural conditions. Each mesocosm presented one bioplastic format to the bacteria, being non-aged biobased polylactic acid (PLA) pellets, PLA-Knife, poly-hydroxybutyrate (PHB) pellet, and PHB-Bag. The study differentiated 3 fractions in the bacterial communities: those free-living in the matrix, those living particle-attached to seawater particles and bioplastic-attached bacteria.

The taxonomical structure of the bacterial community was not conditioned by the bioplastic material, neither the format, but is specific for each of the fractions studied. Bioplastic-attached bacteria are dominated by *Pseudomonadales* and *Phycisphaerales*, as bioplastic-attached specialists, and *Caulobacterales*, *Planctomycetales*, *Flavobacteriales* and *Burkholderiales* as generalists, that also predominate in free-living and particle-attached populations across mesocosms. Commercial format increases abundance of bioplastic-attached bacteria, but it decreases bacterial production and abundance in free-living populations. Our work suggests a negative effect of commercial formats in free-living marine bacteria, leading to a very inactive and scarce population of taxa that are adapted. The taxa contributing the most to the differentiation between fractions will be identified in further analyses. This study will describe the specialization of bacterial populations due to exposure to bioplastics and leaching compounds in coastal surface seawater communities. Further research includes clarifying the chemical compounds responsible of the bacterial community structure after exposure, revealing their role as toxic or energy source compound in the marine environment.

#### **2.05.A.T-03 Bubbly behaviours: physiological and behavioral responses of the sea urchin *Arbacia lixula* from a volcanic CO<sub>2</sub> vent**

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A substantial amount of carbon dioxide released into the atmosphere from human activities is absorbed by oceans and seas, leading to a gradual reduction in ocean pH—a phenomenon known as ocean acidification (OA). OA has been recognized for its influence on the biology of various marine species, particularly those with calcifying properties. However, many studies examining the effects of OA on the physiology and behavior of marine organisms have been limited to aquarium settings, lacking the complexity of ecological processes that interact with changing environmental variables.

In this context, carbon dioxide vents serve as natural laboratories for studying the long-term effects of OA on marine species. This study focused on investigating the physiological and behavioral impacts of chronic OA exposure on the sea urchin *Arbacia lixula*, inhabiting the vents of Ischia, Italy. Adult sea urchins were collected from two vent sites: S1 (pH 8.1; representing ambient pH conditions) and S2 (pH 7.7; representing acidified pH conditions). The experimental design involved reciprocal exposures of adults from both S1 and S2 sites to seawater collected on-site, under both ambient and acidified conditions. Physiological rates (respiration and excretion) and behavioral traits (righting and sheltering time in response to external stimuli) were measured.

The results uncovered a persistent impact of the vent area on the respiration rate of mature sea urchins, particularly notable during the second and third sampling periods. The excretion rate was influenced by pH conditions only in October. Both pH and vent area factors influenced behavioral aspects consistently across all temporal replicates, with a significant interaction effect on righting time. These findings underscore the necessity for long-term observations and field experiments to unravel the intricate processes influencing the physiology and behavior of marine organisms in a changing climate.

#### **2.05.A.T-04 Effects of Ocean Acidification on two calcifying species from the CO<sub>2</sub> vent systems of Ischia Island: *Mytilus galloprovincialis* vs *Patella caerulea***

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CO<sub>2</sub> vent systems, which are naturally acidified sites by the volcanic CO<sub>2</sub> emitted from the seafloor, represent natural laboratory where it is possible to study the effects of Ocean Acidification (OA). Although several studies already demonstrated the adverse effects of OA on marine biota, there are some species that are able to survive and thrive under low pH conditions. This is well exemplified at the Castello Aragonese CO<sub>2</sub> vent systems (Ischia Island), where few calcifiers are present, showing the ability to survive even in the most acidified areas (pH < 7.4). However, the mechanisms underlying the ability of these species to cope with OA are still poorly investigated.

Therefore, in this study we collected specimens of *Mytilus galloprovincialis* (early recruited) and *Patella caerulea* (long-term resident) along the pH gradient of the Castello Aragonese vent (8.1 – 7.7 – <7.4) and from a nearby control site, named San Pietro (ambient pH: 8.1), in order to investigate the biochemical, molecular, and physiological mechanisms that allow survival and that may be involved in acclimation/adaptation. To do so, (i) morphometric and shell decalcification characteristics were measured; (ii) biomarkers related to oxidative stress, energy metabolism and neurotoxicity, and physiological traits were assessed; and (iii) untargeted metabolomics analysis was performed.

Both species displayed heavy shell corrosion as a function of pH level, whereas only organisms of *P. caerulea* exhibited significant variation of their dimension, with an increase of both shell length and soft-body weight in low pH sites. Furthermore, *M. galloprovincialis* exhibited a significant downregulation of aminoacids, nucleosides, lipids, sugars and several osmolites and a significant decrease of glycogen content in organisms from the extreme acidified site compared to those from ambient pH. Conversely, *P. caerulea* mainly showed an upregulation of metabolites, with a significant increase of carnitine and its metabolites, suggesting a potential induction of  $\beta$ -oxidation to sustain the cellular energetic request in response to OA. Finally, both species displayed slight oxidative stress conditions.

Our findings highlighted quite different effects of OA on the two calcifying species, with more detrimental consequences on *M. galloprovincialis* than *P. caerulea*, which could be locally adapted, but further studies are necessary to broaden the current knowledge on the mechanisms that promote tolerance to OA.

### **2.05.A.T-05 Effects of Long Exposure to Different Heatwaves in 2 Reef-forming Species through a Comparison of a Multi-biomarker Approach**

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Anthropogenic stressors negatively affect marine organisms, with sessile benthic species being particularly vulnerable, posing significant risks to marine ecosystems. One of the most widespread threats to reef-forming species is rising temperatures. Heatwaves (HWs), characterized by regional warming anomalies, are rising globally, with the Mediterranean Sea experiencing a rapid increase in frequency, magnitude, and severity. This study presents a comparative analysis of the prolonged impacts of HWs on two key reef-forming species: *Astroides calycularis*, the Mediterranean orange coral, and *Ficopomatus enigmaticus*, an alien and invasive tubeworm. A long-term exposure of 28 days with two distinct HWs ramps was established.

Using as models organisms sessile benthic bioconstructor species known to suffer from HWs-related mass mortality events, and a multi-biomarker approach, the aim is to highlight shared patterns in the biochemical response of habitat forming exposed to different forms of HWs. Parameters assessed included cell membrane damage, cellular reserves, metabolic activity, oxidative status, and biotransformation.

The results demonstrated a negative influence of increased temperatures on the biochemical health of both species studied, with a particular lowering of the protein quantity. Despite differences in shape in the two HWs ramps and the two different species investigated, the biomarker results revealed shared patterns in the response of biobuilders to thermal stress. These commonalities in response to climate anomalies, regardless of organisms or HWs characteristics, suggest a wide-ranging impact of increased temperature. Considering the expected increase in marine temperatures in the near future, further investigations are essential to clarify the effects of this stressor on marine organisms, particularly on benthic sessile reef-formers, which are crucial for maintaining biodiversity in their habitats.

### **2.05.B Marine Ecotoxicology: Impacts and Possible Solutions, From Coastal to Deep-Sea Ecosystems**

#### **2.05.B.T-01 Structural effects of the protein corona formed on various nanoparticles using the coelomic fluid of the sea urchin *Paracentrotus lividus***

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Engineered nanomaterials and nanoparticles (NPs), with one or more dimensions in the range of 1-100 nm, have become widely present in everyday products, raising concerns about their potential nanotoxicity on the environment and human health. Upon dispersal in the environment, NPs tend to interact with biomolecules, particularly proteins. The NP-protein interaction results in the formation of the so-called protein corona, which plays a key role in mitigating or stimulating ecotoxicological responses. In this regard, the study of protein corona and the study of their structural changes are crucial steps to clarify the environmental safety of NPs and facilitate regulatory measures governing their use. To date, studies have mainly focused on the effects of NPs and the related protein corona on humans, neglecting their effects on aquatic species including marine ones.

In our study, we explored how different NPs, varying in size and core compositions such as titanium dioxide (nTiO<sub>2</sub>), functionalized polystyrene (PS-NH<sub>2</sub>; PS-COOH), and silver (AgNPcitLcys), undergo distinct changes in protein corona composition when incubated with the coelomic fluid (CF) of the Mediterranean sea urchin *Paracentrotus lividus*. DLS parameters such as hydrodynamic diameter, polydispersity index and Z-potential, revealing significant alterations in NP surface charges and size during incubation in both CF and NSW. The protein corona composition of different NPs was assessed through SDS-PAGE and LC-MS. Furthermore, Circular Dichroism spectroscopy was employed to evaluate modifications in the secondary structure of the most abundant protein in the CF, the toposome. Preliminary findings suggest that protein corona characterization revealed distinct protein compositions in the various NPs analyzed, several of the most abundant proteins being involved in the sea urchin immune system. Initial results from Circular Dichroism spectroscopy indicate significant alterations in the distribution of secondary structure elements after 12h of NP exposure in the CF of the sea urchin under all analyzed conditions. This study aims to bridge the knowledge gap in the literature regarding the characterization and changes experienced by protein corona when in contact with NPs. Additionally, it lays the groundwork for a future assessment of the ecotoxicological implications these NPs may pose to *P. lividus* and the surrounding environment.

#### **2.05.B.T-02 Emerging Stressors in Deep-Sea Environments: from Microplastics to Deep-Sea Mining**

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The deep-sea is the largest ecosystem on Earth that, despite its remoteness, is also under anthropogenic stressors. Among them, deep-sea mining and microplastics (MPs) are rising as emerging threats. MPs are pervasive in aquatic ecosystems being found in rivers, coastlines, oceans and deep-sea environments. MPs are small plastic particles (<5 mm) that can be ingested by invertebrates, turtles, fish, birds, and marine mammals and eventually by humans through the food web. Deep-sea mining refers to the extraction of mineral resources from the seabed that could, among other effects, release large plumes of sediments across the water column and affect vast areas of the ocean at all levels of the water column. As deep-sea mining-related plumes can also potentially resuspend MPs settled on the seabed, the present work aimed at assessing the effects of suspended sediments and MPs on several benthic species (*Mytilus galloprovincialis*, *Spisula solida* and *Adreus fascicularis*) under hyperbaric conditions through the screening of functional, biochemical and molecular endpoints to better understand how suspended sediments and MPs impact exposed species and the marine environment. Sediments and MPs were used in three different sizes and concentrations to better represent a realistic scenario of resuspension as a mining byproduct. Experiments lasted for 96h at different pressures (up to 50 Bar) in a hyperbaric chamber. After each exposure experiment, several endpoints were analyzed for each species (i.e. respiratory and filtration rates, oxidative stress biomarkers and transcriptomic profiles). Overall, suspended sediments and MPs caused significant detrimental effects in all three species, varying from a filtration/respiratory capacity decrease to changes in oxidative stress response (catalase, lipid peroxidation and glutathione S-transferase) and gene expression profiles. The results of this study show that suspended particles, i.e., sediments and MPs, caused adverse effects on exposed organisms. These findings emphasize the need to establish guidelines and regulations before deep-sea mining starts to mitigate potential negative effects, especially for benthic communities.

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#### **2.05.B.T-03 Oxidative Damage Induced by Aquatic Contaminants in the Critically Endangered Brazilian Guitarfish: Comparison at Different Life Stages**

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Maternal transfer is a process that exposes elasmobranch embryos to a variety of aquatic contaminants during critical life stages. The extent of the consequences of prenatal exposure, especially compared to adults, is unidentified. Hence, this information is crucial for accurate assessment of the contaminant effects in elasmobranchs. Therefore, we aimed to compare the biochemical alterations related to oxidative damage in whole embryos and liver of mature females of the Brazilian guitarfish *Pseudobatos horkelii* facing exposure to metal(loid)s and organic contaminants. Polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs) were assessed through gas chromatography coupled to mass spectrometry, whereas metal(loid)s were quantified using atomic absorption spectrometry with graphite furnace. Lipid peroxidation (LPO) and protein carbonyl group (PCO) levels were also quantified and used as biomarkers. The relationships between embryos and females were assessed using the Principal Component Analysis (PCA). Generalized Linear Models (GLMs) were applied to investigate effects of contaminants on biochemical parameters. In addition, a Permutation Multivariate Analysis of Variance (PERMANOVA) was carried out to evaluate the effect of contaminants and life stage on overall oxidative damage. The PCA results characterized embryo and female samples according to their contaminant levels and biomarkers. Females showed higher concentrations of contaminants whereas embryos were majorly contaminated with PBDEs (BDE-47 in particular). Biomarkers also characterized these two groups, with LPO influencing the female cluster, and associated with Fe concentration. In embryos, PCO was correlated to BDE-47. The GLM failed to detect effect of contaminants on biomarkers. However, when both LPO and PCO were considered together, PAHs, PBDEs, Cd, Cr, Cu, and Fe concentrations significantly influenced oxidative damage in embryos and adult females. The relationship between oxidative damage and contaminant load was not significantly different between these two groups, suggesting that contaminants effects on biomarkers analyzed are similar in embryos and adults. This is of particular concern as early life stages are generally

accepted as being more sensitive to contaminants. Therefore, we conclude that impacts of prenatal exposure to contaminants are comparable to those found in adult guitarfishes.

#### **2.05.B.T-04 BIOACCUMULATION AND DIETARY BIOACCESSIBILITY OF MICROPLASTICS AND COCONTAMINANTS IN MEDITERRANEAN MUSSELS**

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Microplastics (MPLs) are contaminants of emerging concern (CECs) ubiquitous in aquatic environments, where they can bioaccumulate entering the food chain.

In this study, the accumulation of polyethylene (PE), polystyrene (PS), and polyethylene terephthalate (PET) microplastics (MPLs) of sizes inferior to 100 µm was assessed in Mediterranean mussels (*Mytilus galloprovincialis* spp). Moreover, the potential of mussels to bioaccumulate other organic contaminants, such as triclosan (TCS) and polyfluoroalkyl substances (PFASs), was evaluated with and without the presence of MPLs. Finally, the potential human bioavailability of co-contaminants in the presence and absence of MPLs was evaluated by an in-vitro experiment that simulated human digestion. Exposure experiments were carried out in 15 L marine microcosms through the diet. The bioaccumulation of PE, PS, PET, and co-contaminants was assessed by liquid chromatography coupled to high-resolution mass spectrometry (LC(SEC)-HRMS), and the results showed how PFASs were more available when those are adsorbed on plastic surface.

#### **2.05.B.T-05 Beyond the Surface: Investigating UV-Filter Presence and Biomagnification in Three NE Atlantic Pelagic Species**

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The increasing concern about protection against sunrays by health institutions together with the increase in tourism, resulted in the release of vast amounts of sunscreen into the marine environment in the last years. These products contain organic UV filters responsible for protecting against sun rays. Their effect on biota as endocrine disruptors (EDs) have been described in some kinds of species [1], [2], [3] and due to their physical-chemical properties, they are persistent in the ecosystem and tend to be bioaccumulated and biomagnified in organisms throughout the trophic chain being considered Emergent Contaminants [4]. This work aims to evaluate and compare the presence of 11 oUV-F (Table 1.) in shallow and deeper waters, using two epipelagic species, *S.colias*, *T.picturatus* and one bathypelagic specie, *A. carbo*, as a bioindicator. Furthermore, check their potential to be biomagnified through the trophic chain. The extraction process was carried out by optimized Microwave-Assisted Extraction method for 10 minutes at 60°C for the posterior analysis by HPLC-MS/MS. For more information, check [5].

Out of the 11 UV filters analyzed, 6 (4MBC (20.95±11.38), HMS (8.84±2.94), OC (2.90±1.46), UV360 (10.55±7.38), OD-PABA(0.49±0.18)) were detected and identified for *A.carbo*; 3 (HMS (12.23±4.79) UV360 (1.47±0.78), EHS (6.32±2.41)) for *Trachurus picturatus*, and 2 (UV360 (1.14±0.37) and EHS (3.78±0)) for *Scomber colias* (Figure 1.). Higher values are for *Aphanopus carbo*, which is the species that occupies a higher trophic level and is strictly carnivorous. The other two species are zooplanktivorous. The values obtained for the Biomagnification factor (BMFs) indicated that they are potentially biomagnified through the food chain (Figure 1.). The exception was with HMS, which is more abundant in *Trachurus picturatus* than in *Aphanopus carbo* and was non-detected for *Scomber colias*, possibly due to the distribution of this chemical through the water column.

As conclusions, the first results of organic UV-filter concentration in pelagic NE Atlantic species with commercial interest, *T.picturatus* and *A.carbo*, were described in this work.

The biomagnification of this kind of compound is a concern for human consumption, with the higher positions of the trophic chain accumulating more contaminants than those in the lower levels.

The first evidence of the presence of these contaminants in bathypelagic zones using *A. carbo* as a bioindicator.

#### **2.05.P Marine Ecotoxicology: Impacts and Possible Solutions, From Coastal to Deep-Sea Ecosystems**

##### **2.05.P-Tu113 Won't Somebody Please Think of the Lobsters? A Methodological Framework for Toxicity Testing of Pesticides Across American Lobster Life Stages.**

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Infestations by the parasitic sea lice are a challenge to most Atlantic salmon farming regions of the world, including Atlantic

Canada. Annually, approximately 75% of the active marine finfish aquaculture sites in Canada use at least one drug or pesticide to mitigate effects on production performance.

Administration of anti-sea lice products are commonly done through water-bath or in-feed treatments. The former type of treatment involves the usage of chemicals such as azamethiphos (AZA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), among others. Examples of in-feed anti-sea lice products include emamectin benzoate (EMB) and ivermectin (IVM).

Properties of the chemicals influence their fate once released in the environment. Water-bath treatment products remain in the water column affecting planktonic organisms, whereas the in-feed products accumulate in the sediment becoming available to benthic organisms.

In this presentation, we discuss the framework of methods applied to the American lobster (*Homarus americanus*) to understand the potential impacts of bath and in-feed treatments across its entire life cycle.

We performed a series of toxicity tests to produce hazard concentrations of the 4 compounds using various life stages based on different exposure scenarios. Acute exposures to AZA and H<sub>2</sub>O<sub>2</sub> were performed with the planktonic Stage I. Stage IV (first benthic stage) was used in chronic exposure to sediment and feed pellet spiked with EMB or IVM in 10 to 28-day trials. For both Stage I and Stage IV, immobilization and mortality were assessed daily.

Further investigations on the effects of EMB were carried out on: i) the behaviour of Stage IV+ lobsters after 10 days of exposure; ii) intra and transgenerational impacts after 10 days of exposure of adult ovigerous females.

The American lobster proved to be a sensitive and valid model organism for laboratory testing of pesticides.

AZA and H<sub>2</sub>O<sub>2</sub> caused mortality at concentrations well below the application rate but greater than the proposed EQS for these compounds. However, results must be considered carefully, due to dilution effects and degradation of the compounds.

EMB and IVM LC50 values are various orders of magnitude higher than the highest value measured in the environment. Similarly, in both the behavioural assay and ovigerous adult trials, EMB concentrations tested were up to 28 times higher than in the environment and did not cause significant adverse effects.

#### **2.05.P-Tu114 Toxicity of the Scrubber Washwater on Marine Organisms: a Focus on the pH Reduction**

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The emissions of sulphur dioxides (SO<sub>x</sub>) from the maritime transport is a concern for the ecosystems and human health. For this reason, the International Maritime Organization (IMO) establishes limits for the sulphur content in the fuel used by ships. An alternative to the use of more expensive low sulphur fuel is the installation of exhaust gas cleaning systems (EGCSs), known as scrubbers. Scrubbers use water to absorb the SO<sub>x</sub> from the exhaust gas and then this washwater released into the marine environment. Due to the absorption of the SO<sub>x</sub>, the washwater has low pH of approximately 3.8. In this regard, the discharge of scrubber washwater may pose a threat for the aquatic organisms.

In this study, the toxicity of acid washwater was evaluated using three marine organisms as indicators: the bacteria *Vibrio alginolyticus*, the diatom *Phaeodactylum tricornutum* and the crustacean *Artemia franciscana*. The organisms the exposed to mixtures of seawater with simulated scrubber in different ratios, and the lethal or growth inhibitory effect was determined.

The results obtained reported EC50 values of the 37.7% for *V. alginolyticus*, 86.4% for *P. tricornutum* and 95.8% for *A. franciscana*. These results show that the different marine organisms have different sensitivity to the low pH and the dilution level is a major factor in the determination of the toxic potential of the discharged washwater.

The toxic effects by the discharged washwater in the marine organisms imply that, although the use of scrubbers for removing SO<sub>x</sub> from the exhaust gases may help to reduce the impacts of the atmospheric emissions, it poses a challenge for the protection of the marine environment.

#### **2.05.P-Tu115 MicroTox Bioluminescence Assay Sensitivity to Metals Toxicity in Full Strength Marine Water: Implications for Monitoring Anthropogenic Impacts in the Marine Environment**

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MicroTox in vitro bioluminescence assay with *Aliivibrio fischeri* has been proposed for near real-time monitoring of toxicity in marine waters receiving deep ocean metal mining effluent. However, testing of background marine water samples (35 ppt)

against the 2% NaCl diluent provided for the MicroTox assay consistently yields a 20 – 40% increase in microbial metabolic activity and thus light emission relative to the control. This increase in bioluminescence, likely associated with the change in osmotic pressure, serves to mute detection of subtle toxic effects. As a result, 35 ppt pH 7.5 synthetic seawater (SSW) proved more appropriate for use with marine samples. The SSW diluent consistently yielded 10 – 20% increases in light emission following the 15-min exposure period, matching those of field-sourced marine samples whereas 2% NaCl diluent yielded approximately 20% reductions in light emission after the incubation period, explaining the cumulative observed difference of 30 – 40%. Metals toxicity (CuSO<sub>4</sub> and ZnSO<sub>4</sub>) was investigated by testing dilution series produced in each diluent. Resulting dose response curves and calculated EC<sub>50</sub>s indicate near identical sensitivity of the MicroTox assay to CuSO<sub>4</sub> at the two salinities, but significantly reduced sensitivity to ZnSO<sub>4</sub> at the higher marine salinity. These toxicity measures were then compared with *Rotifera* toxicity via Marine RotoTox kits and traditional inland silverside (*Menidia beryllina*) 7-d larval toxicity tests. The RotoTox test was performed in SSW and 2% NaCl diluent to determine salinity effects on metals sensitivity for comparison with MicroTox results. MicroTox proved more sensitive to zinc suggesting it would be protective of marine biota but was significantly less sensitive to copper suggesting that marine biota may still be at risk. In mining effluents we found similar toxicity results across *Menidia*, Rotifer, and MicroTox tests suggesting that MicroTox can give a comparable result for test organisms of interest, though individual metal contaminant constituents should still be considered.

#### **2.05.P-Tu116 Adverse Impacts of Biofilm-Colonized Microplastics on Marine Copepods, *Tigriopus japonicus***

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Microplastics (MPs), as typical emerging contaminants, have caused global environmental concerns with the increase in plastic production. Different types of MPs, can be ingested by zooplankton and cause several adverse impacts, including altered feeding behavior, developmental and reproductive toxicity. The ecological risks that MPs pose to marine organisms have been widely reported, yet there are still knowledge gaps regarding the adverse impacts of environmentally relevant MPs especially caused by colonized biofilm. The aims of this study were twofold: (i) to examine alterations on the physical, chemical, and biological characteristics of 10 µm polystyrene microspheres (PS-MPs) caused by covered biofilm; (ii) to investigate the long-term impacts of biofilm-covered PS-MPs at two environmentally relevant concentrations (100 and 1000 particles/mL) on marine copepods, *Tigriopus japonicus* over a 21-d exposure. Results showed that aggregation occurred for colonized PS-MPs. The oxygen-containing functional groups of PS-MPs increased after colonization with the occurrence of C-OH and C=O functional groups identified by infrared spectroscopy. Colonized PS-MPs selectively enriched Proteobacteria and Bacteroidota in natural seawater, accounting for 78.1% and 16.6% respectively. The biofilm formation significantly increased the uptake of PS-MPs by copepods, and the bioaccumulation of colonized PS-MPs was significantly higher than that of pristine PS-MPs, leading to an increase in bioavailability and chronic toxicity on development and reproduction. This project would provide scientific basis to ecological risk assessment of microplastics in the real marine environments. Further studies on the influence factors of their bioavailability and toxic effects at tissue and cellular levels should be explored for MPs under environmentally realistic multi-stressors.

#### **2.05.P-Tu117 Developmental Effects of Legacy and Novel Type 2 Diabetes Therapeutics to Embryo-Larval Red Drum (*Sciaenops ocellatus*).**

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Current global land-use trends show increasing rates of urbanization in coastal areas, leaving estuaries and other coastal ecosystems particularly vulnerable to the impacts of municipal contaminants. Pharmaceuticals and their by-products are among these constituents of greatest potential concern, due to a global increase in their production and use, in combination with their low effect concentrations. Compounds that target highly conserved pathways are of particular concern to non-target aquatic wildlife, particularly vertebrates. Type 2 Diabetes and obesity are on the rise globally, as are the environmental presence of therapeutics used to treat these disorders. Because metformin has historically been used as the first line therapeutic for Type 2 diabetes, it is routinely measured in surface waters and is one of the highest drugs by weight detected in aquatic environment, along with its metabolically-active degradation product, guanylurea. Both metformin and guanylurea are known to adversely affect the growth and development of several species of freshwater fish; however, far less is known about the potential risk these compounds pose to marine fish. Recently, multiple Glucagon-like Peptide 1 Agonists, including semaglutide (Ozempic) and Tirzepatide (Mounjaro), received FDA approval for the treatment of Type 2 diabetes. However, off-label applications (e.g., weight loss) for these compounds have rapidly gained popularity, as has the use of compounded salt derivatives. The environmental presence and fate of these emerging compounds remains largely unknown, as do potential ecotoxicological risks to non-target aquatic biota in near shore systems. To investigate the relative potency and effects of both legacy and novel therapeutics used in the treatment of Type 2 diabetes, we exposed embryo-larval red drum (*Sciaenops ocellatus*), an economically and ecologically valuable fish species found in the Gulf of Mexico, to equal concentrations of metformin, guanylurea, semaglutide, and tirzepatide. Developmental effects, including changes in morphological endpoints were subsequently evaluated for each of these compounds, to determine whether effects are conserved in marine species.

#### **2.05.P-Tu118 Validation of a miniaturized multiresidue method for the determination of pharmaceutical products in Zebrafish embryos**

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Pharmaceuticals and their metabolites are being found in the environment because of the wastes from laboratories, hospitals and agriculture but also because of their excretion after being consumed. These compounds are considered to be contaminants of emerging concern (CEC) due to their potential toxicity. Toxicity studies are necessary to evaluate the potential risk for the marine biota caused by the presence of these compounds. Zebrafish (*Danio rerio*) embryos are frequently used as model organisms in ecotoxicological studies, and therefore the development of an analytical method for the analysis of pharmaceuticals in this matrix is necessary. The aim of this work is to develop a rapid and sensitive multi-residue method for the determination of pharmaceuticals in zebrafish embryos. The developed method consists of a solid-liquid microextraction (300 µL of solvent) assisted by sonication, followed by centrifugation. This method is adequate for the small sample size (10 embryos). Determination was performed by injection of 100 µL of extract in the liquid chromatograph coupled to triple quadrupole mass spectrometer (LC-MS/MS (QqQ)). The proposed method allows the determination of 52 pharmaceutical compounds including antibiotics, NSAIDs, cardiovascular drugs and psychiatric drugs among others obtaining good accuracy and precision for samples above about 10 pg/embryo in a short period of time, making this method suitable for routine analysis.

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### **2.05.P-Tu119 An integrated approach to determine the food-chain transfer and ecotoxicological effects of UV filters on marine organisms**

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UV filters are considered emerging pollutants due to their widespread use and application; they are used extensively in personal care products such as sunscreens and cosmetics to protect against photoaging and photocarcinogenesis. However, despite their ubiquitousness, very little research has focussed on the ecotoxicological consequences of these compounds, particularly on marine organisms.

Adopting a multidisciplinary approach and incorporating innovative technologies and methods, the research will evaluate the ecotoxicological effects of organic and inorganic UV filters on marine organisms at different levels of organisations (i.e. molecular, cellular and individual levels). The study will adopt marine food-chain analysis and will elucidate the mechanisms of food-chain transfer. The potential biological impact of the pollutants both alone and in combination with UVA radiation will be assessed.

Determination of toxicity potential of UV filters in marine algae (e.g. cellular viability, chlorophyll fluorescence, growth inhibition), potential sub-lethal biological responses in mussels and copepods will be determined (e.g. DNA damage, expression of key genes involved in DNA repair process, histopathological changes), determination of the influence of UV filters on concentrations of organic sulphur compounds (e.g. DMS/DMSP) in the algae will be carried out using purge and trap and GC/MS methodologies. Feeding experiments will be conducted to study the effect of trophic level transfer of UV filters. The bioaccumulation pattern in algae and mussels will be determined using analytical techniques (e.g., GC-MS/ICP/MS).

It is hypothesised that the organic and inorganic UV filters assessed bioaccumulate at different trophic levels in the marine food chain and will result in toxicological effects on the organisms.

This research will highlight the effects UV filters pose to aquatic life, aiding in the awareness of UV-filters as pollutants. UV-filters are emerging chemicals of concern and have already been deemed highly persistent by EU REACH Directives. This research will assist in developing our understanding of the interaction these chemicals have with the marine environment. Overall, the research will fill important knowledge gaps that can be communicated and shared with academic and stakeholder communities. This research is still ongoing, thus, the current results will be discussed and the expected outcomes of ongoing experiments will be highlighted.

### **2.05.P-Tu120 Seasonal concentrations of organic contaminants of legacy and emerging concern on plastic polymers deployed in the Galician coast**

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Plastics act as passive samplers of organic and inorganic contaminants from the surrounding environment (air, water, soil, sediment, etc) and they can be used as an integrative matrix for these contaminants in the marine environment. Additionally, plastics can act as a dispersal vector for their constituents (plastic additives) which can be desorbed and leached to the surrounding environment during physical disintegration processes. Therefore, the analysis of the contaminants sorbed on them can offer an integrated view of chemical pollution particularly for chemicals with medium/high hydrophobicity, more representative than spot seawater analysis. Previous studies have confirmed the concentration of organic contaminants in beached and floating plastics without any information about the exposure period and conditions for the different samples analysed.

In this study supported by 'PLAS-MED' (CICYT, CTM2017-89701-C3) and 'MICROPLASTIX' (JPI OceansProgram, MCIN PCI2020-112145) projects, the seasonal occurrence and distribution of current-use pesticides (triazines, organophosphates and others), personal-care products (fragrances, UV-filters,...), pharmaceuticals, plastic additives, polycyclic aromatic hydrocarbons (PAHs) and organochlorinated contaminants were characterized in deployed plastics in Ares Ria (A Coruña, Spain). Ten different polymers were used (including polyethylene, polypropylene, polystyrene, tyre and others). The sorption dynamism of different pollutant groups was studied sampling at 30, 60 and 90 days of deployed in the surface water of Ares Ria in spring, summer, autumn and winter.

PAHs, UV-filters, synthetic musks and plastic additives were the most frequently detected contaminants, but other personal care products and current use pesticides were also found. It was also observed the potential contribution of plastic additives and other contaminants from polymer formulation to the environment (i.e.: such as PAHs in tyres samples). The individual contaminants found at the highest concentration were octocrylene, hexylcinnamal, and tris-2-chloropropyl phosphate (UV filters and plastic additives). Along the considered period concentrations varied depending on the variations in the predominant pollution sources, mainly urban discharges influenced by tourist, agricultural, livestock, industrial and maritime activities.

#### **2.05.P-Tu121 Looking for Biological Monitoring Tools: Using *Anemonia sulcata* as Bioindicator Species to Assess Sunscreen and Ultraviolet Filter in Temperate Seas**

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Sunscreens contain organic and/or inorganic UV filters (UVF) that absorb, reflect or scatter UV light, inactive ingredients such as antimicrobial preservatives, moisturisers and anti-oxidants. Their use contributes to the release into coastal waters of UVF. Benzofenona-3 (BP3) has been identified as the main organic UVF of concern. It has been reported to induce acute, developmental and reproductive toxicities to different organisms. Some studies have shown that sunscreen ingredients promote viral infections in bacteria and zooxanthellae, causing coral bleaching. Nevertheless, most of these studies are performed using no realistic environmental concentrations of sunscreens and UVF. Different cnidarians, coral species, have resulted to be potential bioindicator species for warm sea ecosystems. In the present study, temperate sea abundant cnidarian species is tested as a potential bioindicator species for the assessment of the exposure and effect of environmental concentrations of sunscreen and BP3.

We aim to determine the suitability of the sea anemone, *Anemonia sulcata*, as bioindicator species using a battery of exposure and effect biochemical biomarkers: oxidative stress, genotoxicity, and bleaching. To achieve this, two independent 7 days-exposure experiments under laboratory conditions were conducted. In the first exposure assay (BP3 assay), individuals of *A. sulcata* were exposed to different concentrations of BP3 (0 to 5000 µg/L). Simultaneously, in the second toxicity assay (sunscreen assay), individuals were exposed to different dilutions (0 to 100%) of a commercial sunscreen containing BP3.

The preliminary results showed a clear and significant bleaching effect with increasing BP3 exposure concentration and sunscreen content ( $p < 0,05$ ) together with zooxanthellae photosynthetic pigments and membrane integrity lose. Although biochemical responses in anemones tissues showed significant activation of defence mechanisms against oxidative stress after exposure to BP3 compared with control treatment ( $p < 0,05$ ), no significant differences with control treatment were observed after exposure to different dilutions of sunscreen.

This work provides relevant information on the potential use of the cnidarian *A. sulcata* as bioindicator species to exposure to UVFs and sunscreen in temperate seas.

#### **2.05.P-Tu122 The impact of the newly approved anti-sea lice drug, imidacloprid, on the polychaete *Capitella* sp**

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The expansion of Atlantic salmon aquaculture in Norway has raised concerns about its impact on the marine environment,

including negative effects on wild salmonid populations, the release of nutrients and chemical pollutants, as well as the spread of parasites such as the sea lice. To control the sea lice infestations in the salmonid farms, strict regulations have been put in place. In order to comply with these regulations, the industry has relied on veterinary medical products (VMP). One such VMP used to combat sea lice infestations is Ectosan Vet, which has as active ingredient the neonicotinoid imidacloprid (IMI). IMI is known to be a neurotoxin that can disrupt the central nervous system of insects and has been linked to the decline of pollinators. While outdoor use of IMI has been banned in the EU, restrictions in other regions, and recognition as an emerging contaminant of concern, there are concerns about its impact on marine biodiversity even at permitted concentrations. The accidental release of untreated IMI into the marine environment has raised further alarm. Wild non-target populations, living in the vicinity of aquaculture facilities are especially at risk of being exposed. However, there are significant knowledge gaps regarding the effects of IMI on the Norwegian marine environment, as studies have predominantly focused on terrestrial and freshwater species. The current study exposed the polychaete *Capitella* sp, a relevant species for the Norwegian marine ecosystems, to Ectosan Vet.

This study examines both lethal and sublethal endpoints in polychaetes collected from the wild. The organisms were exposed to concentrations ranging from 20 mg/L to 0.2 µg/L of IMI, mirroring treatment and release concentrations in salmon aquaculture. The exposure period lasted for 96 hours, followed by a 24-hour recovery period in clean seawater. Throughout the experiment, mortality and immobilization were recorded. Additionally, oxygen consumption rates (OCR) and burrowing behavior were documented as sublethal endpoints for all treatment concentrations. Given that IMI has been shown to induce paralysis and alter mobility behavior in other invertebrates, our hypothesis suggests that the burrowing behavior of polychaetes is negatively impacted. OCR serves as a direct measure of metabolic activities within the organisms, and it is anticipated in this study that increasing concentrations of IMI will result in lower oxygen consumption in the study animals.

#### **2.05.P-Tu123 IMPORTANCE OF THE INFLUENCE OF POPULATION HABITAT ON THE ECOTOXICOLOGY ASSESSMENT OF SUNSCREEN PRODUCTS: AN ANALYSIS USING SEA URCHIN (*P. LIVIDUS*) FERTILISATION AND LARVAL DEVELOPMENT BIOASSAYS**

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Susceptibility of species to different stress agents could be influenced by their habitats. In this sense, phenotypic plasticity could be crucial for marine biota to cope with pollution and, therefore, decisive in environmental risk assessment of contaminants, especially those of emerging concern such as sunscreens. In this study, we investigate the effects of three commercial sunscreens on the fertilization and larval development of two distant populations of the sea urchin *Paracentrorus lividus* located in anthropogenic impacted and pristine coastal areas, respectively. For that, three commercial sunscreens, with different chemical formulas were selected: sunscreen A with “conventional” UV filters (e.g. zinc oxide-nano, octinoxate, and octocrylene), and sunscreens B and C, labelled by the brand as “ocean respect”, with “new generation” UV filters (e.g. ethylhexyl triazone, bis-ethylhexyloxyphenol methoxyphenyl triazine). A range of 10 different concentrations (0 to 500 mg L<sup>-1</sup>) were tested. For the three selected sunscreens, no significant differences were observed in fertilization rate between populations. However, significant differences in larval development were observed regarding the origin of the population. Higher and significant malformations on larval development ( $p < 0.05$ ) were recorded in the population located at the anthropogenic impacted coast compared with the population from pristine area (suntan A:  $EC_{50} = 75.1 \times 10^{-2}$  mg L<sup>-1</sup> and  $EC_{50} = 8.91$  mg L<sup>-1</sup>, suntan B:  $EC_{50} = 23.9 \times 10^{-2}$  mg L<sup>-1</sup> and  $EC_{50} = 5.95$  mg L<sup>-1</sup> and suntan C:  $EC_{50} = 7.64 \times 10^{-3}$  mg L<sup>-1</sup> and  $EC_{50} = 9.12$  mg L<sup>-1</sup> for the populations from the anthropogenic impacted and pristine coastal areas respectively). In general, “new generation” sunscreens showed higher toxicity, indicating that more research should be performed in assessing the risk of these products before to be labelled as ecofriendly. Other parameters, such as the application format (e.g. cream, oil or sun milk) of these products may affect their toxicity.

Besides, this study highlights the importance of population habitat as an essential factor to be considered in ecotoxicity studies.

#### **2.05.P-Tu124 Assessment of Chemicals Chronic Toxicity on Corals, by Combining Measurement of Growth Inhibition and Photochemical Response, With the Scleractinian Coral Model *Stylophora Pistillata***

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Increasing ocean temperature and acidification, overfishing, coastal development, and pollution are well known stressors on coral reefs. They may induce coral bleaching, a process by which corals lose their symbiotic microalgae (zooxanthellae). Weakened corals, more susceptible to infectious diseases, show poor resilience from episodic bleaching events. Ultimately, corals may die when these stressful environmental conditions last too long.

Concern about the environmental impact of UV filters on coral reef have been raised, leading to the ban of certain UV filters in sunscreens such as Oxybenzone and Octinoxate (e.g., Hawaii ; Palau islands). To assess this potential impact, we developed a predictive test protocol based on the inhibition of chlorophyll photosynthetic efficiency of the symbiotic micro-algae in the coral species *Stylophora pistillata* (Fel *et al.* Coral Reefs 2019). One criticism of the methodology was that direct impacts that

chemicals may have on the animal part of the holobionte, may be underestimated if the test endpoint was limited to photosynthesis inhibition only. Therefore, we implemented the methodology by measuring the coral nubbins weight in the sea water [as described by Jokiel et al (1978)], to estimate the impact on growth and mineralization under a chemical stress.

To assess the method feasibility, we exposed nubbins of the scleractinian coral *Stylophora pistillata* for 35 days, using 2 reference compounds (Diuron and TBT), 2 UV filters and 2 preservatives. Exposure regime was semi static with weekly solution renewal. Both photosynthetic efficiency of the zooxanthellae and the weight gain were monitored weekly. The methodology and the results obtained will be presented.

#### **2.05.P-Tu125 Environmental realistic concentrations of octocrylene and benzophenone affect fertilization and embryo development of pacific oyster *Magallana gigas***

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Early life stages of bivalves are more sensitive to environmental changes than adults and demonstrate a high sensitivity to various toxic substances. Ultraviolet (UV) filters are among the most commonly detected compounds in water, sediments and biota. They are bioactive, persistent and have a high bioaccumulation potential due to their high stability, lipophilicity, and low biotic degradation. Several studies point to the UV filters harmful effects on marine biota, though information regarding marine bivalves' early-life stages and fertilization is still scarce. Thus, the present study aims to evaluate the effects of two UV filters, octocrylene (OC) and benzophenone (BP), at environmentally relevant concentrations (1, 10 and 100 µg.L<sup>-1</sup>) in pacific oyster (*Magallana gigas*) early life stages through a multiparameter approach by evaluating fertilization rate and D-shape larvae development, swimming activity and DNA integrity. The fertilization assays followed 3 approaches: exposure of oocytes, exposure of spermatozoa, and exposure of both gametes. The exposure duration was 30 minutes, and then fertilization rate was calculated by counting unfertilized oocytes among 100 oocytes. Oyster embryo-larval assay followed ICES (*International Council for the Exploration of the Sea*) guidelines, and then the swimming activity was recorded for each replicate. DNA integrity was assessed in oyster larvae cells through the comet assay. The fertilization rate decreased after oocyte exposure to 10 and 100 µg.L<sup>-1</sup> of OC and BP and after spermatozoa exposure to 100 µg.L<sup>-1</sup> of BP. When both gametes were exposed, the fertilization rate decreased in 1 and 100 µg.L<sup>-1</sup> of OC and 100 µg.L<sup>-1</sup> of BP. All concentrations of OC and BP caused developmental abnormalities in D-shaped larvae (24h). The swimming velocity of larvae exposed to 1 µg.L<sup>-1</sup> of OC and to 10 µg.L<sup>-1</sup> of BP decreased. No larvae DNA damage was found. OC and BP induced a reduction of the fertilization rate, embryotoxicity and a decrease in swimming velocity at environmentally relevant concentrations. These sub-lethal effects may have significant consequences for the survival of oysters' larvae, delaying settlement and increasing the risk of predation, which might lead to a reduction of oyster recruitment.

#### **2.05.P-Tu126 Vulnerability of the Sea Urchin (*Paracentrotus lividus*) to Various Scrubber-waters: Implications for Fertilization and Larval Development**

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In response to sulphur regulation rules approved and adopted by the International Maritime Organization, many shipping companies have invested in scrubber-water systems instead using lower sulphur fuels. This decision was taken without any risk assessment of the effects of scrubber-water on marine and coastal ecosystems. This way, the exhaust gases containing SO<sub>x</sub>, NO<sub>x</sub>, CO<sub>x</sub>, Polycyclic Aromatic Hydrocarbons (PAHs), alkylated PAHs, metals and particles were sprayed with seawater to remove the atmospheric pollutants and transferred them to the seawater. An extremely acidic toxic effluent was created containing potentially persistent compounds with carcinogenic and mutagenic effects. This scrubber-water is continuous and directly discharged into the surrounding surface water.

The present work aims to investigate the ecotoxicological effects of scrubber-waters on the most sensitive stages of development in marine invertebrate organisms, specially focusing on fertility and larval development of the sea urchin *Paracentrotus lividus*. Given that sea urchin releases the gametes directly into the water, the seawater quality impacts embryonic development.

Three types of scrubber-water produced with Atlantic seawater were used: scrubber-water A (SWA) artificially produced, and Scrubber-water B and C (SWB, SWC) produced on board two different ships. A range of scrubber water concentrations (0.001 to 100% v/v sea water) were tested either with or without pH correction. The scrubber-waters were analysed, revealing notably low pH values and an enrichment of PAHs and alkylated-PAHs, as well as trace metals. The exposure of *P. lividus* spermatozooids and embryos to scrubber-waters evidenced concentration-dependent effects on fertilization success and on the larval development, with the latter proving to be a more sensitive bioassay. The assays performed with pH correction, exhibit lower toxicity, which can be explained by the decrease in metal's bioavailability. The current results indicate that scrubber-waters are highly toxic to this invertebrate species, posing a threat to the more sensitive larval stages crucial for species

recruitment and the sustainability of the entire marine food webs. These bioassays have proven to be effective in assessing the risks posed by scrubber-waters to marine aquatic ecosystems.

#### **2.05.P-Tu127 Biomarker Responses of Clams (*Ruditapes philippinarum*) Fed on Bisphenol A Analogs-Contaminated Food**

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Bisphenol A (BPA) analogs are currently used in manufacturing and as plasticizers as a substitute for BPA. This replacement is taking place because BPA is recognized to be an endocrine disruptor chemical (EDC) that can also cause oxidative stress and genotoxic effects. BPA analogs are speculatively considered safer compounds than BPA and their usage is increasing with a consequent increasing environmental release. In this study, we fed specimens of the clam *Ruditapes philippinarum* for 7 and 14 days with microalgae previously exposed to an environmentally relevant concentration of 300 ng/L of the three main BPA analogs: BPAF, BPF, BPS and their mixture (MIX) to allow their bioaccumulation into the microalgae. Effects on several biomarkers indicative of cytotoxicity and oxidative stress and damage were evaluated in target tissues, such as haemocytes, gills and digestive gland. Results showed that the ingestion of microalgae growth in a BPA analogs-contaminated environment can cause alterations of total haemocyte number and their proliferation. In addition, the activity of phosphatases was significantly altered in consequence of the contaminated microalgae ingestion. Regarding the oxidative stress, we observed an alteration of both antioxidants responses in both gills and digestive gland. Similarly, we observed an increased oxidative damage on lipids and proteins. These results suggest that BPA analogs can be harmful compounds for clams even through a dietary intake and more effort should be performed to assess the effects of these compounds in marine food chains.

#### **2.05.P-Tu128 Combined effect of polystyrene microplastics and bisphenol A on the embryo development of the sea urchin *Arbacia lixula***

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Microplastics (MPs, <5 mm) can act as environmental vectors or sinks for hydrophobic organic pollutants, such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons, pharmaceuticals, and perfluorinated surfactants. Until now, the toxic mechanisms of contaminant mixtures are not well understood in marine organisms. In this context, the toxic effects of polystyrene MPs (PS MPs, 1 and 5 µm; 10 µg/mL) and bisphenol A (BPA; 5 and 25 µM), alone and in combination, were herein evaluated on the embryo development of the sea urchin *Arbacia lixula*. Treatment with the two concentrations of BPA significantly reduced the normal larval development, showing skeletogenic alterations at 5 µM and blocked development at 25 µM. On the contrary, both sizes of PS MPs did not significantly reduce the normal embryo development of sea urchins, while the morphological changes observed following co-exposure of the two sizes of PS MPs and the two concentrations of BPA, resemble those obtained following treatment with BPA alone. The impact on nervous system (serotonergic, cholinergic, GABAergic), stress response, energy metabolism and biomineralization process was therefore investigated through immunohistochemical, molecular and metabolomic analysis to understand the mechanism of action of PS MPs, BPA, and their combination. Overall, these studies provide useful information about the toxicodynamic of complex mixtures of contaminant such as PS MPs and BPA during the embryogenesis of *A. lixula*, which is confirmed to be a sensitive model system for emerging stressors. Furthermore, these findings could contribute on the understanding of the impact of combined PS MPs and BPA on the health of marine organisms living in heavily polluted coastal areas.

#### **2.05.P-Tu129 Risk Characterization of the Antifouling Biocide Tralopyril and Two Natural Compounds Produced by Cyanobacteria: Fertilization and Embryotoxicity Tests using *Magallana gigas***

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In the past decades, several biocides have been restricted in the European Union under the framework of the Biocidal Products Regulation (EU Regulation No. 528/2012). Antifouling (AF) biocides are not exception and thus, there is a plea for environmentally acceptable alternatives. Natural compounds with AF activity have been identified as possible alternatives to AF systems. Nevertheless, their natural origin alone is not guarantee of environmental safety. In this sense, this study aims to expand the information available on the toxicity of two natural substances produced by cyanobacteria, with AF potential, namely portoamides and nucoulin A (NocA), and compare their results with those of tralopyril, an AF biocide accepted by the BPR. Therefore, in this work we investigated the toxicity of tralopyril, portoamides and NocA against fertilization and larvae development of the oyster *Magallana gigas*. Furthermore, the comet assay was also used to assess the genotoxic potential of these three substances on *M. gigas* D-larvae. Preliminary results point towards a higher toxicity of the commercial AF biocide tralopyril when compared to portoamides. On the other hand, NocA seems to have a similar toxicity regarding larvae development but much lesser effect regarding fertilization. Additional data will be assessed and discussed in order to evaluate the genotoxic potential of the assessed substances.

#### **2.05.P-Tu130 Development of green coating solutions for the problems of maritime corrosion and biofouling**

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Marine biofouling and corrosion are two substantial challenges for shipping, offshore and aquaculture infrastructures, and maritime technologies exposed to sea water. Expensive corrosion resistant materials are often needed to ensure long term equipment functioning while antifouling solutions are required as surfaces and ship niche areas are typical hot spots for the accumulation of biofouling organisms. For these reasons, environmentally friendly and more cost-effective solutions to protect these niche areas offering a combination of corrosion and biofouling protection of metal surfaces are needed.

Effective, environmentally safe corrosion and biofouling protection can be potentially achieved through the investigation and use of high-tech nanomaterial-based formulations, and the development of a thin coating with functional antifouling and anti-rust additives in a green and eco-friendly final product that can be tested in a newly developed innovative testing unit. A novel dual purpose anticorrosion and anti-rust coating solution under development by the PRONICARE project aims to achieve these goals by:

-investigating a wide range of copper-free green coating formulations with functional additives, through ecotoxicological testing on species of different trophic levels: bacteria, microalgae, microcrustaceans, and fish, and also on two invasive marine biofouling organisms

-developing a new mobile test unit and methodology to validate the effectiveness of the coating mimicking real world environmental conditions and maritime niche areas.

-conducting environmental impact and life cycle analysis of the coating.

#### **2.05.P-Tu131 Assessing the Impact of New Emerging Nanomaterials in the Mussel *Mytilus edulis***

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The fast expansion and use of nanotechnology in science and industry has continuously led to the development of new emerging nanomaterials (NMs) with unique properties and diverse applications but unknown impacts in the environment. Layered nanostructures, for example, have shown significant promise for use in environmental technology and remediation due to their specific physicochemical properties. This is the case of defect-rich molybdenum disulfide (MoS<sub>2</sub> NMs) and layered double hydroxides nanosheets (LDH NMs), that have been explored for effective removal of contaminants from water samples. However, the impact of these new emerging NMs on aquatic ecosystems are still poorly understood, as well as their potential toxic effects in organisms. Mussels *Mytilus sp.* are widely acknowledged organisms to assess the toxicity and environmental risk of NMs. Thus, the present study aimed to assess and compare the toxicity of MoS<sub>2</sub> and Mg-Al-LDH NMs towards the mussel *Mytilus edulis*. Mussels were exposed to both NMs at 0.1 and 1 mg/L for 14 days, followed by 7 days of depuration. A wide range of biomarkers were determined in mussels at different time points (0, 7, 14 and 21 days), as well as bioaccumulation in tissues. These included condition index, lysosomal membrane stability (LMS), micronuclei formation, DNA damage, acetylcholinesterase activity, antioxidant enzymes activity, lipid peroxidation, cellular energy allocation and metallothionein levels. MoS<sub>2</sub> and Mg-Al-LDH NMs behaviour in exposure media was also evaluated using Transmission Electron Microscopy and Dynamic Light Scattering. Analyses are underway, but preliminary results seem to point to a significant effect in exposed mussels that is concentration and time dependent, with Mg-Al-LDH NMs presenting a clearer toxic response than MoS<sub>2</sub> NMs. This is evidenced by the results obtained on the LMS, where a lower retention time was observed in mussels after 7 and 14 days of exposure to 1 mg/L Mg-Al-LDH NMs and after 14 days for 0.1 mg/L. For mussels exposed to MoS<sub>2</sub> NMs, a significant decrease was only detected for 1 mg/L after 14 days of exposure. Lysosomal retention time returned to values similar to the control after the 7 days of depuration for all exposed mussels. Overall, this study provides valuable information on how new emerging NMs could become a potential risk for the aquatic environment and organisms. This work was supported by the SCANNER Project (#299261) funded by the Research Council of Norway.

#### **2.05.P-Tu132 Approaches to Standardized Methods for Identifying the Negative Influence on the Biodiversity of Coral Ecosystems.**

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It is very apparent that climate change significantly affects most ecosystems resulting in potentially dramatic changes of food webs. Further chemical pollutants affect ecosystems in a significant way. One of the most endangered ecosystems are coral reefs. Up to now, special focus has been given to the effect of skincare chemicals on corals. But there is still a knowledge deficit in identifying and quantifying other risks. Increasing run-off of pesticides from agriculture and industrial wastewater are origins for contaminants in the coastal water. Ecotoxicological research is useful for assessing potential risks and simulating impacts under laboratory conditions. Building new test systems that can quantify potential risks to the environment is an important tool for avoiding biological breakdown in a multicomplex system. We are currently working on new test methods to

evaluate ecotoxicological risk for coral reefs. We present test approaches for the generation of reliable and comparable toxicity endpoints.

### **2.05.P-Tu133 The impact of chemical stressors on biodiversity - An integration of environmental forensic techniques and biological effect markers in the blue mussel *Mytilus edulis***

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Anthropogenic activities, including habitat fragmentation, overfishing, freight transportation facilitating invasive species, and pollution events, pose a significant threat to biodiversity. Human population growth contributes to resource overexploitation and chemical pollution. Diverse ecosystems exhibit higher resilience, making them better equipped to withstand environmental stressors. While traditional monitoring methods focus on population and biological effects, there's a lack of integration with the effects of pollutants on biodiversity. Monitoring biodiversity changes due to chemical pollution in the marine environment is challenging with traditional methods, that require high working effort. However, eDNA metabarcoding emerges as a sensitive, non-invasive and cost-efficient tool for detecting a broad taxonomic range. This study integrates eDNA, chemical profiling, genetic and biochemical studies and compares them with traditional monitoring methods. The goal was to establish a simple integrated monitoring strategy applicable in yearly campaigns. The focus is on correlating biodiversity results from eDNA with biological effect monitoring using the mussel species *Mytilus edulis*.

Sediment and mussel samples were collected from two polluted sites on the Swedish Baltic Coast and one reference site. RNA extraction and cDNA synthesis were conducted on six individuals from each site. Quantitative PCR was performed using primers for genes encoding glutathione S-transferase (GST), multidrug resistance-related protein (Mrp2), and antimicrobial peptides mytilin and myticin. Target gene expression was normalised to the reference gene 18S rRNA. Preliminary findings indicate higher antimicrobial peptide gene expression in one polluted site (Ronneby harbour) compared to the reference site Torhamn. Notably, mussels from the second polluted site Karlskrona, a busy marine traffic area with a naval base, exhibit the lowest expression of all target genes. Ongoing analysis includes eDNA, chemical and metal assessments with integration of these results planned for early 2024.

The impact of chemical pollution on biodiversity has the potential to bring about profound and lasting changes in crucial marine ecosystems. This initiative is a dedicated step towards minimising such events, contributing to the advancement of biomonitoring strategies. The focus is on optimising and streamlining monitoring procedures, aiming to reduce costs and efforts, while enhancing efficiency.

### **2.05.P-Tu134 Testing Marine Heat Waves: physiological and behavioural tolerance in a population of the seurchin *Paracentrotus lividus***

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The warming of seawater and the occurrence of marine heatwaves (MHWs) pose a treat to marine organisms, playing a significant role in the fragmentation and loss of coastal marine habitats. It is crucial to comprehend the resilience and potential adaptation of species to ocean warming to better predict future changes and enhancing current conservation strategies. This study conducts a thermo-tolerance experiment to explore the physiological and behavioural effects of short MHWs on the seurchin *Paracentrotus lividus*, an ecologically and economically relevant species in different area of the Mediterranean Sea and Atlantic Ocean. Adult individuals, collected from the Lagoon of Venice (Italy) in early October 2023, were initially separated by gender through the examination of genital papillae. The separation was conducted using a stereomicroscope. Subsequently, five females and five males were placed in each of six independent tanks, each with a volume of approximately 50 liters. The tanks were filled with natural seawater and maintained under a water flow of 200 ml/min, exposed to natural light conditions. To simulate the HW temperature was progressively increased from 20 °C in the first 3 days until the temperature of 28 °C was reached. This temperature level was then kept for 3 days and then restored to ambient level. Hence, the entire exposure lasted for 9 days. After that, behavioral responses of sea urchins were evaluated. Each individual was tested in terms of: 1) "covering time", i.e. the ability to cover itself with *Ulva* sp. blades; 2) "sheltering time", i.e. the speed with which it escapes from artificial light to shade itself; 3) "righting time", i.e. ability to rotate from "upside-down" to "upside-up" position. Ultimately, the respiration rate of each individual was assessed utilizing optic fiber technology. No significant alterations were observed in any of the tested responses following short-term exposure to heat-wave conditions. These results highlight the behavioral and physiological plasticity of this *P. lividus* population from the Lagoon of Venice, a highly variable environment, to heat-waves' events. This experiment took place at the facility of the Hydrobiological Station Umberto D'Ancona at the University of Padova with the involvement of students from the Marine Ecotoxicology course within the Master Degree in Marine Biology of the University of Padova (Italy), that are listed among the authors.

## 2.05.P-Tu135 Investigating primary producers buffer effect against mercury pollution in the sea urchin *Paracentrotus lividus*

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Over the past few decades, human activities have significantly altered and deteriorated marine environments by releasing various contaminants. The oxygen release by coastal photosynthetic organisms holds promise for alleviating environmental perturbations, enhancing marine animals' aerobic performance in challenging conditions, as observed for ocean warming and acidification. However, our knowledge of potential buffering effects in response to pollution is still scarce. This understanding becomes pivotal for predicting future changes and enhancing current conservation and restoration strategies of primary producers-based habitats. This study investigates the potential buffering effect against mercury pollution by examining increased oxygen concentration, simulating primary productivity. The sea urchin *Paracentrotus lividus*, an ecologically relevant species in the Mediterranean Sea and the Atlantic Ocean, serves as the model organism. Adult *P. lividus*, collected from the Venice Lagoon, were exposed to the combination of 2 level of oxygen saturation (90% as control and 160% representing daily supersaturation) and 2 concentration of mercury (0 and 1 mg/L) for 7 days. At the end of the exposure, coelomocytes related biomarkers, individual respiration rate and behavioural traits were measured in exposed sea urchins. Results show that righting time was significantly influenced by the interaction of the 2 tested factors with sea urchins exposed to the presence of mercury in a hyperoxygenated condition faster than those from the other 3 conditions. Furthermore respiration rate was significantly influenced by hyperoxygenation with an increased metabolism of exposed sea urchins. This experiment took place at the facility of the Hydrobiological Station Umberto D'Ancona at the University of Padova with the involvement of students from the Marine Ecotoxicology course within the Master Degree in Marine Biology of the University of Padova (Italy), that are listed among the authors. The study was conducted as part of the RETURN Extended Partnership, funded by the European Union Next-Generation EU (National Recovery and Resilience Plan – NRRP, Mission 4, Component 2, Investment 1.3 – D.D. 1243 2/8/2022, PE0000005).

## 2.05.P-Tu136 Investigating primary producers buffer effect against mercury pollution in the clam *Ruditapes philippinarum*

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Marine coastal areas are strongly affected by global change and environmental restoration activities with the reintroduction of primary producers are commonly carried out. However, the presence of contaminants in seawaters, like heavy metals, even in restored areas, poses some questions. Indeed, some studies highlighted that a higher presence of primary producers with a consequently increased oxygen concentration in seawaters can have a buffer effect on the toxicity of contaminants, leading to a reduction of physiological and biochemical alterations in animals. For this reason, we investigated if the presence of primary producers can have a buffer effect on the toxicity of mercury or, on the contrary, if there is enhanced toxicity of this heavy metal on clam specimens. In detail, we mimicked the presence of primary producers by exposing the animals to hyperoxia condition (160% of oxygen saturation) with respect to the specimens maintained in normoxia condition (90 % of oxygen saturation), while the mercury was added at an environmentally realistic concentration of 1 µg/L. The exposure lasted 7 days and there were four different conditions: normoxia/no Hg; hyperoxia/no Hg; normoxia + Hg and hyperoxia + Hg. After the week of exposure, we measured the total haemocyte count and the mean diameter and volume of haemocytes. In addition, we investigated the presence of cytotoxic effects between the tested conditions. The results showed that the total haemocyte count was not altered by both mercury presence and oxygen concentration. In the opposite way, we observed that both the mean diameter and volume of haemocytes were significantly reduced by 1 µg/L of mercury. However, the presence of a higher oxygen concentration was not able to restore the initial haemocyte size. Interestingly, we did not record an increased cytotoxicity in any of the treatment conditions. In conclusion, we did not observe a buffer effect on mercury toxicity caused by the simulation of a higher presence of primary producers (hyperoxia condition) which means that the reduction of pollutant concentrations in seawaters still appears as the main way to reduce their hazard. This experiment took place at the facility of the Hydrobiological Station Umberto D'Ancona at the University of Padova with the involvement of students from the Marine Ecotoxicology course within the Master Degree in Marine Biology of the University of Padova (Italy), that are listed among the authors. The study was conducted as part of the RETURN Extended Partnership, funded by the European Union Next-Generation EU (National Recovery and Resilience Plan – NRRP, Mission 4, Component 2, Investment 1.3 – D.D. 1243 2/8/2022, PE0000005).

### **2.05.P-Tu137 Does it pass? And what does it do? Maternal transfer and consequences of pollutants' accumulation in sharks**

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Sharks are among the most threatened groups of vertebrates. Due to their longevity and high trophic level, these species accumulate high pollutant levels, yet the threat represented by pollution is still highly overlooked. The few information available focus on risks for humans that consume shark meat, rather than on the negative consequences for the animals. Similarly, virtually no data exist on maternal transfer for most of the species; nevertheless, embryonic and early life stage exposure to pollutants might represent a severe risk for health, growth, and survival. This research aims at investigating pollution levels and maternal transfer in three common viviparous shark species inhabiting the northern Adriatic Sea (Mediterranean Sea), *Squalus acanthias*, *Mustelus mustelus* and *M. punctulatus*, and correlating these levels with potential health and physiological consequences. Samples of muscle, liver, blood, placenta, gonads, and embryos have been collected soon after capture from males and females, mature and immature individuals, and pregnant females found among the landings of commercial vessels. Heavy metals, persistent organic pollutants (POPs), and emergent contaminants, such as pharmaceuticals and personal care products (PPCPs), will be quantified in all tissues. Biomarkers associated with pollutant exposure and potential negative effects for the animals will be investigated, including biomarkers of oxidative stress, cytotoxicity, metal exposure, reproductive impairments (i.e., egg quality), alterations to immune function, and general health/energetic status. Maternal transfer will be confirmed and quantified comparing maternal and embryonic pollutant levels. Potential negative effects of pollutants' accumulation will be investigated analysing the correlation between biomarker levels and pollutant concentrations. Multivariate analysis will highlight whether different pollutants act synergistically in causing homeostasis/health impairments. The observed results will be fundamental to better understand the vulnerability towards pollution of these already threatened species, highlighting the potential need for the introduction of effective conservation measures.

### **2.05.P-Tu138 Scope for Growth in the Blue Mussel *Mytilus edulis*: Effect of Paint Leachates from Offshore Wind Farm**

Moses Ndugwa, Biology, University of Antwerp, Belgium

Moses Ndugwa<sup>1</sup>, Katharina Alter<sup>2</sup>, Bavo DeWitte<sup>3</sup>, Lieven Bervoets<sup>1</sup> and Gudrun De Boeck<sup>1</sup>

Wind energy is part of the clean energy systems promoted to minimize the environmental impacts of fossil fuel combustion for power generation. Offshore wind farms have recently gained popularity for several reasons, such as sufficient wind speed and less spatial competition compared to on-land wind farms. Additionally, offshore wind farms present great opportunities for co-location of aquaculture farms alongside electricity generation. However, biofouling coupled with corrosive properties of seawater calls for proactive solutions to minimize the deterioration of offshore wind farm installations, instigating the use of paints mainly composed of epoxy resins and polyurethanes. Despite their application, little is known about the ecotoxicological effect of paints on marine life. Paints used in offshore wind farms installations may leach potentially toxic chemical compounds such as, derivatives of bisphenols, tributyltins, benzophenones and per- and polyfluorinated substances, which may negatively affect marine biota. In laboratory experiments, we are investigating the scope for growth of the blue mussel following exposure to paint leachates from offshore wind farms. Thereby, the mussels will be positioned either directly on the paint coupon (direct exposure) or in close proximity to the paint coupon (indirect exposure). Oxygen consumption rate, maximum clearance rate, and ammonia excretion will be assessed after 1, 3, 7, 14, and 21 days of exposure. Our current preliminary results after 3 days exposure showed an indication of some effects of the paint used in offshore wind farms on the scope for growth in *M. edulis*. Overall these results underscore the importance of understanding the ecological impact associated with the use of such paints in offshore wind farms. This knowledge sheds light on the potential environmental risks posed by rapid global expansion of offshore wind farms.

### **2.05.P-Tu139 Improving the Survival of Copepods in Ecotoxicology Trials**

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*Ecotoxicity Studies in Copepods*: Copepods are ideal candidates for ecotoxicity studies due to their ecological relevance, distribution across a range of environmental conditions, culture within small systems, and sensitivity to pollutants. This has encouraged their wide use within marine ecotoxicology studies, their proposal as a marine model species for OECD assays, and their establishment within ISO assays for both acute and early life stage toxicity. However, all stages of the life cycle after hatching are prone to physical damage which can lead to mortalities occurring days after the initial injury, thereby jeopardizing assays following ISO methods which stipulate the transfer of animals into test solutions. In this study, novel techniques were used to avoid transferring of live animals which resulted in increased welfare and improved data. These steps can be easily integrated into wider ecotoxicological applications in the genera.

### **2.05.P-Tu140 Electromagnetic fields from subsea power cables: A risk driver for biodiversity changes of offshore wind?**

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Offshore wind farms (OWF) utilize subsea power cables for intra-array and export purposes, generating electromagnetic fields (EMF), comprising electric (E-fields) and magnetic fields (B-fields). Electroreceptive marine species, like certain sharks and rays, with specialized organs, may be affected by E-fields, impacting their ability to locate prey. B-fields are likely to disrupt geomagnetic cues and navigation in species relying on such cues, altering natural behaviour and navigation patterns. The



impact on marine organisms depends on field strength, species sensitivity and cable proximity to sensitive habitats. Cable insulation and burial can mitigate environmental impact, with burial being more effective. EMF strength varies with cable voltage and current, where B-fields are current dependent, and E-fields are voltage dependant. HVDC cables, responsible for transmitting electricity to the onshore grid, are high voltage, cover longer distances, and are seabed-buried. Benthic and epibenthic communities, critical for maintaining the ecosystem functionality, are thus at risk of EMF exposure. Some benthic invertebrates, like lobsters and crabs, are sensitive to B-fields, which causes behavioural alterations. The overall impact on organisms, communities and ecosystems remains poorly understood and studied. SINTEF Ocean developed an experimental system for controlled exposure of marine organisms to B-fields. The system, featuring an exposure arena, allows observation of small marine organisms while subjecting them to realistic magnetic fields from submarine cables. The primary research focus is assessing behavioural effects on various marine species, aiming to establish EMF effect thresholds. This research contributes to evaluating EMF as a potential environmental risk factor for offshore wind installations.

#### **2.05.P-Tu141 Strategic Investigative Drivers and Impacts Affecting Marine Environmental Developments**

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*Aquaculture*: The importance of aquaculture is only too evident in light of unsustainable harvests from capture fisheries. A sustainable need for the sector is apparent in order to meet future projected demands for aquaculture production to fulfil global food production demands exacerbated by rapidly growing human population and climate change issues. Aspects of pertinence impacting aquaculture sector production and sustainability include:

global temperature rise; ocean acidification; disease & harmful algal blooms; change in precipitation (rainfall) patterns; sea level rise; uncertainty of external input supplies; changes in sea surface salinity and severe climatic events.

The multi-faceted and site-specific nature of the environmental impacts of aquaculture mean that the primary route for managing these impacts is through the assessment, including through modelling, of individual projects or plans taking into account relevant regulatory systems.

*Aquatic Ecotoxicology*: Ecotoxicology research and developments are important for the assessment of well-known and emerging environmental contaminants. The conservation of ecosystems and human health is based on a sound assessment of the risks associated with the presence of contaminants in the aquatic environment. In reference to the use and issues of ecotoxicologically relevant species key criterion is the extrapolation of studied species to the whole ecosystem. Important factors for consideration are: (1) the genetic diversity of organisms used in bioassays and their extrapolation to wild populations; (2) criteria to be taken into account for biomonitoring - this is pertinent to the structure and functioning of the whole ecosystem; (3) the variability of responses through taxonomic levels and (4) the variability of responses as a result of confounding factors.

In context of aquatic environment a natural process of significant concern for many industries is biofouling. Biofouling is undesirable biological accumulation of organisms (micro- & macro- organisms) and other constituents on surfaces exposed to natural waters. This biogrowth causes operational inefficiency imposing substantial cost for the maritime and aquaculture industries. Perspective to tackle & address these important aspects will be conveyed.

#### **2.05.P-Tu142 "Unveiling the Long-Term Effects: Exploring Multigenerational Impacts of Commercial Sunscreens on the Marine Microalgae *Phaeodactylum Tricornutum*"**

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The expanding knowledge about the danger of sunburn, in relation to photoaging and skin cancer, has led to a notorious increase in the use of sunscreens around the world. However, these cosmetics are considered pollutants of emerging concern, especially in marine ecosystems. Sunscreens are composed of an extent range of chemical compounds, making them complex matrices to assess from an environmental point of view. Despite of the number of studies evaluating the detrimental effects caused by the ingredients released from sunscreens on marine environment, they have been focused on the short-term responses of a given species, ignoring how marine biota will respond to this new anthropogenic invader when taking into account trans-generational plasticity

The main objective of this study is to evaluate the effects of commercial sunscreens in the marine microalgae *Phaeodactylum tricornutum* through consecutive generations. The working hypothesis is that sunscreens may not represent a danger for the development of microalgae at short but across generations. To achieve this goal, experiments with *P. tricornutum* were carried out in triplicate. Exponentially-growing populations of microalgae were exposed to five different concentrations of a mixture of five commercial sunscreens (15, 30, 60 and 90 mg L<sup>-1</sup>) and a control (natural seawater) under laboratory conditions. The experiments were performed during seven consecutive generations with an established initial cell density of 10<sup>4</sup> cells mL<sup>-1</sup>. Cell density, metabolic activity and chlorophyll-*a* content were measured every 96 hours (at the end of each generation). Preliminary results showed at first a positive relationship between all the selected responses and the concentration of sunscreens, but this tendency changed after the third generation, when the cultures started to show cells damage.

The conclusions obtained from this work proportionate valuable knowledge about the trans-generational effects that sunscreen products may cause on microalgae species. This study highlights the need of conduct multigenerational studies to accurately predict how marine biodiversity will respond to the presence of this new contaminant and avoid over or under estimate its effects at long term.

### **2.05.P-Tu143 Ecotoxicological Impacts and Recovery Potential in Deep-sea Anemones Exposed to a Sediment Plume Generated by a Deep-sea Mining Vehicle**

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The deep sea represents 90% of the world oceans and is mostly unknown due to its magnitude and inaccessibility. Recently, humankind started to look to the deep sea as a potential hotspot of mineral resources that will be essential for the expected green energy transition. One of the major challenges, that has barely been assessed, is the potential ecotoxicological impacts from the exploitation of such resources. This study will provide the 1st ecotoxicological results from deep-sea anemones exposed *in situ* (4500m depth) to a plume of sediments in the Clarion-Clipperton-Zone (CCZ), Pacific. The 1st trial of the prototype Patania 2 robot for deep-seabed mining of polymetallic nodules was closely monitored by the Mangan21 expedition in May 2021, that collected *Actiniaria spp.* specimens from control and exposure areas. After 18 months from the trial, further anemone specimens were collected. Metal concentrations (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, V, and Zn) were determined by ICP-MS and biomarkers of oxidative stress (SOD: Superoxide Dismutase, CAT: Catalase, and GST: Glutathione S-Transferase) and damage (LPO: Lipid Peroxidation) as well as Metallothioneins (MTs), were determined with spectrophotometry, both in whole tissues. All metals, except Zinc and Arsenic, were found in higher concentrations on the individual exposed to the plume when compared to the controls. SOD activity seems to be induced in the exposed anemone, similar to LPO, although less pronounced. Results from metal accumulation and biomarkers after 18 months from the impact trial will also be presented to enable an estimation of the recovery potential in this species. Preliminary results indicate that the single deep-sea anemone exposed *in situ* to the sediment plume, accumulated several metals in its tissues, and that may have caused oxidative stress and damage, when compared to control locations. This shows that sediments from the seafloor of the CCZ might have the potential to cause ecotoxicological harm to the deep-sea fauna. This is essential information for the determination of future thresholds to be incorporated in regulations by policy makers to minimize the environmental damage of this activity.

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### **2.05.P-Tu144 Identifying the Best Biomarkers for Risk Assessment in the Deep Sea**

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Deep-sea mineral resources are seen as economically valid and technologically possible to exploit. However, the cost-benefit of deep-sea mining (DSM) need to accurately account for the scale of environmental impact, the ecosystem services that might be compromised and the potential mitigation measures that can be implemented. DSM will remove the habitat locally where the mining operations will take place. In addition, localized plumes created by mining collectors at the seafloor and dewatering ore slurry produced by the mining support vessel may introduce complex mixtures of potentially toxic elements in the water column. While species resilience to impact will be conditioned by their capacity to resist exposure to toxic elements at present it is not possible to predict, in advance of DSM operations, the absolute toxicity thresholds. It is thus important to develop novel methodologies and indicators for ecotoxicological evaluation of risks of metal exposure for deep-sea organisms. This study aims to assess the ecotoxicological impacts using transcriptomics and proteomics approaches and to identify potentially new biomarkers. *In situ* sulfides/sediments exposure experiments with local fauna from deep-sea hydrothermal vents and cold seeps were performed. Mussel samples (*Bathymodiolus azoricus* and *Gigantidas platifrons*) were collected from the Lucky Strike hydrothermal vent field (1700m depth) Mid-Atlantic Ridge and from the Haima seep (1385m depth) in the South China Sea, respectively. In the present study, we suggest potential biomarkers that are representative of different biological systems (immune system, metabolism and oxidative stress) that were modified under those exposure scenarios. Twenty-four biomarker candidates based on high-throughput methodologies results were selected and tested. The validation of candidate biomarkers

will help to identify which are the most reliable. Results will help to define future threshold values for plume / metal concentrations, information seen as essential to be incorporated in regulations by policy makers to minimize the environmental impact of DSM activities. This work was funded by Fundação para a Ciência e a Tecnologia (FCT) through the BiDiRisk (PTDC/CTA-AMB/2894/2021) and DEEP-REST projects (DivRestore/0009/2020), and the grants CEECIND005262017, CIMA UI/MAR/00350/2020, and LA/P/0069/2020 and Fundamental Research Funds for the Central Universities, China (202172002 and 202241002).

### **2.05.P-Tu145 Simulating the deep-sea mining plume exposure on a shallow-water mussel: an ecotoxicological assessment**

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Due to the high metal demand worldwide, the interest in deep-sea mining is increasingly pronounced, mainly for extracting polymetallic nodules, of high commercial value due to their high concentration and purity in metal contents. The Clarion-Clipperton Zone (CCZ) is one of the prominent locations targeted for mining these polymetallic nodules due to its high abundance. During mining on the seafloor, the generation of sediment plumes may release metals in concentrations that can induce ecotoxicological harm. This was investigated on the shallow-water mussel, *Mytilus galloprovincialis*, frequently used as a bioindicator organism. Mussels were exposed for 14 days to concentrations of deep-sea sediment (10 mg/L (C10) and 50 mg/L (C50)) collected from the Belgian contract area of the CCZ. The biochemical biomarkers were evaluated, including those involved in the antioxidant defense system: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPx), xenobiotic biotransformation system: glutathione S-transferase (GST), oxidative damage: levels of lipid peroxidation (LPO), neurotoxicity: acetylcholinesterase (AChE), genotoxicity (comet assay). Elemental screening was performed to detect metals and REE in the mussel's tissues and experiment waters. Results from the exposed waters showed a significantly higher metal (Co, Cu, and Mn) and REE (Ga, Eu, Yb, Dy, Y, Ho, Er, and Lu) content in the C50 treatment, compared to the control waters ( $p < 0.05$ ). Similarly, higher Cr and Mn accumulation was found in the gills of the mussels after 14 days of exposure, whereas in the digestive glands, Mn, Ni, and Co were significantly higher after 14 days of exposure ( $p < 0.05$ ). Regarding biomarker responses, pronounced antioxidants and biotransformation responses were found, namely elicited SOD, GPx, and GST activities, culminating in an absence of oxidative damage (LPO levels) in both tissues after sediment exposure ( $p > 0.05$ ). Signals of genotoxicity and neurotoxicity were also found in mussels exposed to the sediments. Overall, the results of the present study provide evidence of negative ecotoxicological impacts caused by deep-sea sediments on the shallow-water mussel *Mytilus galloprovincialis*.

### **2.05.P-Tu146 Potential Biomarkers to Assess Metal Toxicity in Deep-sea Invertebrates under Deep-sea Mining Scenario**

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Deep-sea mining (DSM) activities will remove extensive accumulations of minerals from the deep ocean and are expected to release potentially toxic metal mixtures through the generation of sediment plumes near the seafloor or from the dewatering ore slurry after the ore process. These plumes may spread and impact larger areas and may disrupt the normal functioning of biological mechanisms (e.g. immune system, metabolism and oxidative stress), adversely affecting deep-sea organisms. The crosstalk between the ecotoxicological effects and specific biomarkers across different deep-sea invertebrates has been less explored using omics promising tools. This review summarizes the existing studies and methodologies in deep-sea marine invertebrates that applied genetic techniques and bioinformatic tools, with emphasis on the high-throughput profiling, to assess environmental impacts at the transcript, protein, and metabolome levels. In the present study, we suggest several potential biomarkers that are representative of different biological systems that were modified under increased metal concentrations in deep-sea invertebrates adapted to very harsh environmental conditions (e.g. high pressure, low temperature and absence of sunlight). Our findings highlight several biomarker candidates that could be screened in priority in future ecotoxicological studies related to DSM. However, those potential biomarker candidates need validation with further experiments simulating DSM plume exposure disturbances with deep-sea organisms. Once validated, these can work as early warning signals for environmental disturbance, especially for the exposure to the toxic metals entrained in DSM plumes. Moreover, the definition of potential threshold values for the best candidate biomarkers may allow future biomonitoring actions during DSM activities. The authors thank to Fundação para a Ciência e a Tecnologia (FCT) through the BiDiRisk (PTDC/CTA-AMB/2894/2021) project, the grants CEECIND005262017, CIMA UI/MAR/00350/2020, and LA/P/0069/2020, and Fundamental Research Funds for the Central Universities, China (202172002 and 202241002).

### **2.06.P New Developments in Sediment Ecotoxicology and Risk Assessment**

#### **2.06.P-We099 Estuarine Sediments as “Traps” for Trace Metals, PAHs and Hormones: An Example from Southampton Water, UK**

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Understanding the fate and behaviour of contaminants across different environmental matrices is crucial for effective risk assessment and management. Estuarine sediments are recognized as "traps" for a range of contaminants, playing an important role in moderating their input from rivers into coastal and marine ecosystems. However, degradation pathways for contaminants in sediments depend on the physicochemical properties of the sediments, such as sediment composition, particle size, organic matter content, the chemical structure and speciation of the contaminants and the environmental conditions. Given the vast amount of different chemicals, and varying environmental parameters, for many contaminants the fate in sediments is still uncertain.

This study focuses on the fate of hormones, PAHs and trace metals in estuarine sediments. Analysis of surface sediment samples and sediment cores from Southampton Water, a heavily industrialized and urbanized estuary in Southern England, allow for a spatial distribution, as well as a historic record of different contaminants in this area. Focusing on three different contaminant groups provides the opportunity to compare the behavior dependent on different chemical properties and moreover, gives a more comprehensive overview of the condition of the estuary. So far, trace metal analysis using XRF analysis indicates increasing concentrations close to point sources such as the port (e.g. Hg with up to 2.4 mg/L), whereas with gamma-spectroscopy dated sediment cores document contamination linked to historical waste discharges into the estuary. Statistical analysis, such as Principal Component Analysis, demonstrates associations between trace metals and the sulfides in the sediments, but no general trends of correlation. Ongoing work includes organic analysis using GC-MSMS. Furthermore, adsorption batch experiments are conducted to gain further understanding of how different chemical structures, as well as varying types of organic matter influence the adsorption and degradation of certain contaminants in estuarine sediments. These results will help to further explain the observations from the environmental sample analysis and contribute to a more comprehensive understanding of contaminant dynamics in estuarine systems.

#### **2.06.P-We100 Paleo-ecotoxicology: Dioxin-like activity in lake sediment core as an indicator of historical anthropogenic environmental pollution**

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The hydrophobic nature of dioxin-like organic substances (PAHs, PCBs, PCDD, and PCDF) leads to the sorption onto organic matter of sediments in aquatic ecosystems, where they can remain stable over extended periods. Sediment cores from lakes play a significant role as a natural archive of pollutant inputs over time. This allows insights into the concentration, composition, origin of the pollutants and the change of those parameters over time.

This study is part of a interdisciplinary historical project that explores the rich history of the town Bad Waldsee in Germany. It focuses on the analysis of ecotoxicological and chemical properties using sediment cores from the Stadtsee lake. The collaboration with historians, geoscientists and botanists within the project, enables to attribute the ecotoxic effects to specific historical events as well as to the ecological data of the lake. The extracted sediment cores display clear lamination, which allows the dating of the core to a (partially) annual resolution over the last 700 years. The aim of this research was on investigating biomarker CYP1A for dioxin-like activity in sediment, cross multiple subsamples of sediment core dated from 2021 AD to 600 BC to record the presence of ecotoxicological pollutants over time. The approach is based on 7-ethoxyresorufin-O-deethylase (EROD) induction in the H4IIE rat hepatoma cell line, with the determination of a Biological Toxicity Equivalent (Bio-TEQ) to TCDD for each sediment subsample. First findings indicate significant variations in the ability of sediment core subsamples to induce EROD activity, with an exceptionally high values detectable during the early modern age (1500 AD - 1800 AD), reaching up to 1000 pg TCDD/g sediment. EROD activity induction in some samples was attributed to specific historical events such as city fires and military conflicts. Data of concentrations of 21 PAHs explained 4.8% to 57.5% of the observed EROD activity in the sediments subsamples. However, EROD activity has been documented since the Middle Ages and steadily increases over the years, suggesting long-term exposure to various dioxin-like compounds. To comprehensively capture components of organic pollutant exposure, specific time subsamples will be further investigated using a non-target analysis. These findings contribute to a deeper understanding of historical environmental pollution and provide valuable insights into development of anthropogenic stressors before industrialization.

#### **2.06.P-We101 When and How to Conduct Ecotoxicological Tests Using Natural Field Collected Sediment**

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In recent years, the sediment compartment has gained more attention when performing toxicity tests with the growing emphasis on gaining more ecological relevance in testing. Though many standard guidelines recommend using artificially formulated sediment, most sediment studies are performed using natural sediment collected in the field. The use of natural

field collected sediment contributes to more environmentally realistic exposure scenarios and higher well-being for sediment-dwelling organisms. This underscores the need for comprehensive guidelines on conducting sediment toxicity testing with natural sediment.

In this method paper, we outline six key steps in conducting ecotoxicity assays with natural field collected sediment: collecting sediment in the field, preparing the sediment, characterizing the sediment, altering the sediment, spiking the sediment, and finally using the sediment in an experimental setup. Drawing from decades-long expertise in sediment testing we propose the following recommendations: 1) natural sediment should be collected at a site, that has been well-studied both historically and by laboratory analyses; 2) to collect and store larger quantities of sediment prior to the initiation of an experiment to ensure a uniform sediment base; 3) any sediment used in ecotoxicological testing should be characterized, at the very least, for its water- and organic matter content, pH, and particle size distribution; 4) to select a spiking method, equilibration time, and experimental setup based on the properties of the contaminant and the research question; 5) to include control-, treated similarly to the spiked sediment, and solvent control sediment when appropriate; and, 6) to quantify experimental exposure concentrations in the overlying water, porewater, and bulk sediment at the beginning and the end of an experiment.

It is important to point out that any handling of intact natural sediment will change its physical and chemical properties. Here, we propose a stepwise method that provides control and consistency while maintaining sediment properties in as natural of a state as possible. This method solely applies to instances where a uniform sediment is preferred *e.g.*, when rearing cultures or performing ecotoxicological investigations where you control or add contaminants. Overall, we hope that by making these steps clear and accessible, we can increase the comparability and reproducibility of studies conducted with natural sediments.

#### **2.06.P-We102 A Comprehensive Examination of Fluoxetine Exposure in *Lumbriculus variegatus*: Insights from Biomarkers to Behaviour**

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Sediments play a vital role in freshwater ecosystems, providing habitat for organisms like decomposers and bioturbators, which are essential for ecological processes such as the uptake and transfer of nutrients. The increasing chemical pollution threatens these sediments, which can act as a sink for persistent organic pollutants such as certain pharmaceuticals. However, there is a considerable knowledge gap regarding the chemical impact on sediment-dwelling organisms such as *Lumbriculus variegatus*. Existing studies assessing chemical effects in *L. variegatus* primarily focus on survival, growth, and reproduction, neglecting biochemical and behavioural responses. However, these additional sublethal endpoints have become more important in recent years as they provide crucial insight into chemical effects. This study addresses this gap by exposing *L. variegatus* to the antidepressant fluoxetine, assessing various endpoints: survival, growth, reproduction, respiration, biochemical responses related to cellular energy (*e.g.* lipids), oxidative stress (*e.g.*, catalase), neurotoxicity (acetylcholinesterase) and serotonin levels as well as behavioural analysis (chemical avoidance, phototaxis, and feeding behaviour).

Two sequential experiments were conducted by exposing *L. variegatus* to fluoxetine through sediment, following OECD guidelines. Six concentrations between 0.0025 and 25 mg/kg were tested in 28-day exposures with five replicates and ten organisms per replicate. Fluoxetine exposure significantly impacted the growth and reproduction of *L. variegatus*. Additionally, worms showed behavioural changes even at low, environmentally relevant concentrations in the phototactic and feeding assay. The chemical avoidance assay showed a significant response at 0.025 mg/kg, with organisms failing to avoid lead contamination, unlike the control. These effects will be linked to the biochemical analysis in a subsequent step.

This study introduces novel approaches for evaluating the impact of exposure on the behavioural responses of *L. variegatus* and links this response to physiological and biochemical effects. The results show that fluoxetine exposure significantly affected the physiological and behavioural responses of *L. variegatus*, even at environmentally relevant concentrations. This indicates that non-conventional endpoints like behavioural assays provide essential tools for chemicals like fluoxetine, designed to affect the hormonal pathways at low concentrations.

#### **2.06.P-We103 Evaluating the use of an *in vivo* bioassay battery for sediment quality assessment in small streams**

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Under the Water Framework Directive (WFD), a tiered approach is recommended for sediment quality assessment. A first tier consists of chemical analyses and the evaluation against sediment quality standards (EnQS). Exceedance of EQS should trigger additional investigations to verify the possibility of potential adverse effects, in particular if the EQS is considered "uncertain". The Technical Proposal for Effect-Based Monitoring and Assessment under the WFD concluded that *in vivo* bioassays could already be considered for implementation. However, a guidance document would be useful to help identifying a suitable battery of tests depending on the type of pressures, and to facilitate the assessment of the results. The Ecotox Centre carried out a monitoring study with the aim of extending the assessment of water quality using effect-based methods. To this end, water quality was assessed at sites with extensive, agricultural or agricultural-urban land use using a comprehensive bioassay battery

of largely standardised water and sediment bioassays. This presentation will provide an overview on the results of sediment bioassays.

The battery of sediment bioassays included the tests with the ostracod *Heterocypris incongruens*, the nematode *Caenorhabditis elegans*, and the insect *Chironomus riparius*. Physico-chemical analyses included sediment properties, metals, and organic contaminants (PAHs, PCBs, and organic substances including 91 pesticides and degradation products). The ecotoxicological quality of sediments was assessed by means of existing toxicity thresholds. Measured concentrations were compared with EQS. Sediments from sites under extensive land use were less toxic than those from agricultural or agricultural-urban land use, which was supported by lower measured chemical contamination. Sites affected by agricultural-urban land use showed the highest potential risk (exceedance of EQS for all substance types), which agreed with a medium or unsatisfactory ecotoxicological quality. Sediments from agricultural watersheds showed medium or unsatisfactory ecotoxicological quality. Only slight exceedances of the EQS for metals, PAHs, PCBs were observed, while several pesticides exceeded the EQS. Our results support the implementation of an in vivo bioassay battery, in particular in areas that are not expected to be at risk because they are located far from known local sources and to take effects of not routinely analysed chemicals into account.

#### **2.06.P-We104 Utilizing Natural Deep Eutectic Solvents for Eco-Friendly Analysis of Chemical Pollutants in Marine Sediments**

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Marine sediments reflect the dynamic interactions between terrestrial and aquatic ecosystems and can be considered archives of environmental processes. Conventional chemical characterization methods often involve hazardous solvents, presenting ecological impact and safety challenges. This study explores Natural Deep Eutectic Solvents (NADES) as an eco-friendly solvent alternative in the extraction of chemical components within marine sediments. NADES are a combination of two or more natural compounds, typically hydrogen bond donors and acceptors, which results in a mixture with unique properties, making them promising candidates for efficient extraction of target compounds. Therefore, they can be applied in the extraction of chemical contaminants from solid samples. This work proposes a novel approach for the simultaneous extraction of inorganic and organic contaminants in marine sediments. The NADES extract is characterized via High-Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) for (ultra)trace inorganic elements, and via Fourier-Transform Infrared (FTIR), Thermogravimetric analysis (TGA), and High-Performance Liquid Chromatography (HPLC) to investigate the presence of organic pollutants. The comparison with traditional extracting methods will be discussed, NADES is expected to preserve the integrity of extracted pollutants during the analytical process. Ultimately, using a one-step extraction can contribute to the development of fast and reliable methods supporting greener and more responsible research practices in marine environmental science.

#### **2.06.P-We105 Metals Distribution in Sediments in Select Creeks in Metro Vancouver, Canada**

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Watersheds in Metro Vancouver, the third largest metropolis in Canada, contain mixes of varying land use including park, residential, commercial, industrial, institutional, and agricultural. Storm water which typically empties directly into rivers or creeks in these watersheds can potentially introduce pollutants such as metals that are picked up as the storm water flows along these areas with varying land use. Forty-seven sediment samples were collected from 23 creeks in Metro Vancouver to assess how the surrounding land use influence metal distribution in the sediments. Total metal concentrations were determined by x-ray fluorescence and the physicochemical properties of surface water were also evaluated. There were no discernible statistically significant relationships between the surrounding land use and surface water quality parameters including the pH (which ranged from 5.5 to 8.27), total dissolved solids (13.5 to 410 ppm) and electrical conductivity (18.9 to 903  $\mu$ S/cm). The pH of some sampled sediments were however lower and correlated with sites with high metal concentrations. The mean concentrations in the sediments were Ag: 1.1, As: 1.9, Ba: 486, Cd: 1.8, Cr: 54, Cu: 28, Fe: 34448, Mn: 915, Ni: 29, Pb: 21, V: 92 and Zn 141 mg/kg. The concentrations of Ag, As, Cd, Cr, Cu, Pb and Zn in sediments from some of the creeks exceeded either the Canadian Council of Ministers of the Environment (CCME) interim freshwater sediment quality guidelines or the threshold effect level. Sediments in creeks closer to highways with heavy vehicular activities contained significantly higher concentrations of As, Cd, Cu, Pb and Zn. Principal component analysis indicated good correlation between these elements corroborating the common anthropogenic origin. Fe, Ni and V were attributed to natural weathering from the geological formation of the study area. Enrichment factors, contamination factors, geo-accumulation indices and pollution load index (PLI) for the sediment samples differed among the land uses. The calculated contamination factors followed the sequence: Cd>Ag>As>Pb>Zn>Mn>Cu>Ba>Cr>Ni>V. PLI exceeding 1 that indicated polluted sediments were noted for four creeks situated in industrial land use catchment areas. Additional investigations including determining the areal extent of contaminants in the identified creeks and the types of industries in the impacted watersheds are ongoing. Seasonal variability of metal levels in the sediments will also be conducted.

## **2.06.P-We106 Organic contaminants in sediment cores from the lake of L'Albufera Natural Park (Valencia, Spain)**

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Sediments of lakes and other shallow waters have been considered in different studies as excellent archives for defining long-term variations of contaminants in the environment. For this reason, records derived from the chemical analysis of sediment cores could be useful to trace the history of contaminant emissions in a selected area. This study is aimed to assess the vertical variation of pharmaceuticals and other organic and inorganic contaminants (OICs) in sediment cores of two different sites from the lake of L'Albufera Natural Park (Valencia, Spain), to ascertain whether these cores are able to provide information regarding the historical variation of the accumulation of contaminants in sediments. A sediment core sampler was used to extract the cores from the lake and then were cut into 7 segments of the same thickness using a stainless-steel cutter. The organic contaminants were extracted using methanol-Mc Ilvain buffer (pH 5.6)-EDTA and the metals using acid digestion on microwave oven, the detection was performed by an Orbitrap Exploris 120 mass spectrometer (Thermo Scientific) and an ICP-MS ICAP6500 DUO (Thermo Scientific), respectively. Organic contaminants were determined both using wide target screening against a positive list of compounds, and non-target screening applying ddMS2 of the 4 more intense ions in each cycle as well as all ions fragmentation. Several organic contaminants, especially pesticides and pharmaceuticals such as azoxystrobin, imazalil, tebuconazole, bisphenol A, butyl paraben, ethyl paraben, salicylic acid and codeine were detected in superficial sediment from 0-10 cm and in the deeper layers. Generally, the higher heavy metal concentrations appear in the more recent sediments (depth < 20 cm), reflecting the increased agricultural and industrial activities in the area during the 20<sup>th</sup> century. This study showed that organic contaminants were lixiviated in different amounts through sediments columns, and their occurrence cannot be directly related with the age of them. However, these sediments can also be used to examine contamination mechanisms, which are significant for predicting future contamination tendencies, and assessing potential environmental risks in important areas as L'Albufera Natural Park.

## **2.06.P-We107 To Peat or Not to Peat, That Is the Question. Exploring Coconut Husk as an Organic Matter Enrichment Alternative in Sediment Toxicity Test.**

*Davide Asnicar and Benjamin Patrick de Jourdan, Huntsman Marine Science Center, Canada*

The utilization of standardized artificial sediment in toxicity assessments offers increased repeatability and enhances consistency across bioassays and laboratories. Artificial sediment is required for most OECD (Organisation for Economic Co-operation and Development) guidelines, particularly in studies assessing the impact of chemicals on benthic communities.

While peat is conventionally incorporated into artificial sediment formulations for toxicological testing, it is not a sustainable resource due to the long time it takes to form and the environmental impact of its extraction. Notably, peatlands, covering over 12% of Canada's land area, are pivotal in carbon sequestration, estimated to be 150-160 Gt of carbon. The extraction of peat leads to a carbon loss equivalent to 2.1 megatonnes of carbon dioxide emissions annually, accompanied by the potential release of other elements into the environment and aquifers. Consequently, the pursuit of less impactful alternatives is desirable, and aligns with the 2030 Agenda for Sustainable Development, endorsed by the OECD.

In the context of sediment toxicity experiments, peat serves as a source of organic matter (OM) in artificial sediment. Here we investigated the viability of coconut husk as an alternative to peat in formulating artificial sediments for toxicity testing. While prior suggestions have been made regarding the use of coconut husk, further research is necessary to ascertain the stability of OM values in sediment over chronic studies and to verify the affinity of the chemical of interest to coconut husk, especially when testing chemicals with diverse characteristics (e.g., different Kow).

Utilizing the Loss on Ignition method, we determine the quantity of coconut husk required to attain equivalent OM content in artificial sediment formulations, mirroring the mass used for peat.

To evaluate the efficacy of coconut husk as an alternative, parallel toxicity tests were conducted using artificial sediments created with peat and coconut husk under identical conditions. The results indicate that the presence of coconut husk does not introduce toxicity effects of the tested chemicals, suggesting its suitability as a viable alternative for toxicity testing.

## **2.06.P-We108 Acute Lumbriculus variegatus Test Assessing Physiological Responses to Chemicals: A Simplification of the Chronic OECD 225 Test**

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With the OECD test guideline No. 225, a well-established chronic exposure description using *L. variegatus* exists, which provides valuable information on the bioaccumulation and reproductive effects of test substances and polluted sediments on the annelid. However, due to the test set-up only a restricted amount of annelid tissue is available and additional biomarker

analysis, providing data on physiological responses of the test organism, is usually not possible. Thus, for such studies, e.g. on the activities of enzymes which are part of the xenobiotic metabolism or oxidative stress responses, a simplified test set-up is needed to allow for large numbers of replicates and the rapid removal of animals throughout the test period.

In this project, we developed a short-term, well-plate exposure set-up without sediment for *L. variegatus* with the aim of analyzing the annelids for biomarkers of physiological reactions to chemical exposures and tested the set-up with Cadmium as a positive control substance. We exposed annelids individually to two concentrations of Cd for up to 96 h and analyzed the activities of Glutathione-S-transferase (GST) and Catalase (CAT), as well as the concentration of Metallothionein (MT) in the annelid tissue at three time points during the exposure. Bioaccumulation was also assessed for each time point, to be able to relate biomarker effects to internal Cd concentrations. The chosen aqueous Cd exposure concentrations were also tested in an OECD 225 chronic exposure study.

We found a significant Cd bioaccumulation in both experimental set-ups. In the short-term study we saw a significant increase of MT concentration after 24 h, 48 h and 96 h of exposure. Additionally, compared to the negative control, the CAT activity of the annelids significantly increased after 48 h of exposure and subsequently significantly decreased after 96 h. Our results match the known potential of Cd to induce oxidative stress, as CAT is part of the defense against reactive oxygen species and hydrogen peroxide, and MT is involved in metal detoxification mechanisms. Our study presents a novel experimental set-up for assessing molecular biomarkers in the common test species *L. variegatus* and provides first data on the validation of this novel method.

### **2.06.P-We109 Beneath the Surface: Exploring Genotoxic Effects of Polycyclic Aromatic Hydrocarbons (PAHs) and Metabolic Responses in *Capitella teleta*, a Sediment Dwelling Species**

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Marine environments face persistent exposure to contaminants, including PAHs with hydrophobic properties making them prone to partition into the sediment. Sediment also serves as the main food source for many benthic species, posing potential risks to these organisms. *Capitella teleta*, for instance, it lives in, and feeds on organic rich sediment. It is a key organism of the benthic community and is therefore selected as a model species to assess its' vital role in the fate of many contaminants including PAHs. *C. teleta* is recognized for its' ability to accumulate PAHs, potentially resulting in biotransformation. *C. teleta* is known to have the capacity of biotransforming various contaminants via Cytochrome P450 monooxygenase (CYP450) and phase 2 enzymes, it is also a distinguished species as it exhibits 96 functional CYP enzymes. Nevertheless, information on biotransformation and effects of PAHs in *C. teleta* is scarce. The aim of this study is to identify & quantify the involvement of major CYP enzymes in the biotransformation of Benzo[a]Pyrene (BAP) via functional and gene-expression assays. In addition, we aim to quantify the genetic damage caused by exposure to BAP. This study also aims to establish a baseline for a future multigenerational study, designed to understand the associated longer-term effects of PAH exposure on *C. teleta*. BAP is a PAH known to be mutagenic and carcinogenic and was selected for this initial phase of the research. The study design followed a 28-day exposure to a range of concentrations (0-150 µg/g wet sediment) in triplicates, with overlaying filtered seawater of 31‰ salinity, and constant aeration. As this study aims to assess genotoxicity, the Comet assay will be used to quantitatively assess DNA damage. Additionally, to understand the biotransformation process of BAP in *C. teleta*, ECOD, a biochemical assay used to measure the activity of a group of CYP enzymes will be performed, in combination with Reverse Transcription Polymerase Chain Reaction (RT-qPCR) to give insights into specific gene-expression levels of various CYP enzymes at different time-points of exposure. Our hypotheses are as follows: 1) BAP may potentially cause genetic damage in *C. teleta*. 2) Chosen CYP enzymes may show an induction & up-regulation in expression as a bio-transforming response to BAP. The findings will also contribute to a broader understanding of PAHs' mode of action in *C. teleta*.

### **2.06.P-We110 To Avoid or Not to Avoid Rare Earth Element Contaminated Sediment? That is the Question for *Daphnia magna***

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There is still little known about the ecotoxicology of the current emerging contaminants Rare Earth Elements (REE). Most conventional ecotoxicity test focus on acute and chronic effects in media, but not in sediment. Our study focused on the potential of how an indirect effect of REE contaminated sediment could possibly have high environmental relevance in the form of active avoidance. The objective was to determine if sediment contaminated with two REEs, lanthanum (La) and gadolinium (Gd), leads to avoidance behaviour by pelagic organisms such as *Daphnia magna*. A round experimental vessel for the horizontal migration and a rectangle vessels for the vertical migration were 3D printed using transparent PETG filament. The vessels contained characterized sediment (Lufa 2.2) and M4 media without EDTA. The sediment was spiked with concentrations in the anthropogenic range of 25; 50; 75; 100 mg kg<sup>-1</sup>, and environmental relevant concentrations of 0.001; 0.01; 0.1; 1; 10 mg kg<sup>-1</sup> for both La and Gd. For each exposure, 3 adult *D. magna* were observed for 15 seconds after 0h, 1h, 2h, and 3h. Scoring of the daphnia was based on the location of the daphnia within the experimental vessel for the duration of the 15 seconds. All tests were repeated at least 5 times. Independent observations were collected by experimenters. The results indicated that REE contaminated sediment caused avoidance behaviour for *D. magna*. However the avoidance is related



of both the specific REE and its concentration. The results indicate that there is perceived stress by the daphnids, resulting in behaviour changes which may affect their energy metabolism and survival rate, e.g. by rendering them more susceptible to predators. The avoidance of REE-contaminated sediment affecting the behavior of pelagic organisms like daphnids could have further implications for the wider freshwater ecosystem.

#### **2.06.P-We111 Assessment of the Toxic, Neurotoxic and Genotoxic Effects of Sediments from the Lower Basin of the Papaloapan River, Veracruz, Mexico.**

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The Papaloapan River is an important system in our country that flows into the Gulf of Mexico. This river receives a continuous supply of toxic compounds due to the industrial, agricultural and livestock activities that take place on its banks. In this study, an evaluation of the presence of compounds with toxic, neurotoxic and genotoxic effects in the sediments of the river bed and associated systems was carried out, to detect the areas with the highest degree of contamination. 10 samples were collected in the final part of the river (18° 18' - 18° 47' N and 95° 44' - 95° 51' W) during the dry and rainy season for 3 years (2018, 2019 and 2022). The following parameters were evaluated from the samples: pH, volatile sulfides, ammonium, organic matter, organic carbon, conductivity and texture. The toxicity of sediments was determined with bioassays (*Daphnia magna* and *Pseudokirchneriella, subcapitata* in fresh water and *Artemia franciscana* and *Tetraselmis* sp. in brackish and marine environments). The evaluation of genotoxicity was carried out with the SOS-Chromotest test and the neurotoxic effect was determined with an *in vitro* assay. The information generated was integrated into a multivariate analysis to establish the degree of contamination of the sediments. The mortality percentages obtained in the toxicity bioassays with the sediments varied from 20 to 68%. The sediments with the highest toxicity were obtained from the areas near the mouth of the river and in the Embarcadero lagoon, in these sites the sediments also had genotoxic and neurotoxic effects. A direct relationship was observed between sediment toxicity and ammonia concentrations and conductivity, and between genotoxicity and organic carbon levels. Likewise, greater sediment toxicity was observed in the dry season. The areas with the highest degree of contamination were Embarcadero lagoon and the adjacent areas to the mouth of the river.

#### **2.06.P-We112 Using Paleotoxicology to Study the Influence of Anthropogenic Compounds in the Last Century**

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Plant protection products (PPPs) are currently a topic of high concern. However, more information is needed about the diversity of these agrochemicals and their actual levels and effects on the environment. As sediment cores provide historical records of past environmental contamination within a catchment, a comprehensive assessment of PPPs and heavy metals in lake sediments can help to unravel valuable information on the long-term effect of anthropogenic compounds in aquatic systems.

In this work, a multiproxy workflow that combines paleolimnological (hyperspectral scanning,  $\mu$ XRF) and paleotoxicological techniques (toxicity testing) and chemical analysis (trace elements, LC-MS/MS) was applied to investigate the impacts of PPPs and trace elements on sediment-dwelling organisms (ostracods) in the last century. This work reveals that sediments of two small eutrophic ponds located in the Bernese Mittelland (Switzerland) and known to be contaminated with PPPs cause toxic effects on sediment-dwelling organisms, namely ostracods. By performing *in-situ* toxicity tests, ostracods were found to show increased growth inhibition and mortality in more recent and older sediment layers of the pond. The obtained results were complemented with the analysis of ostracod fossils, revealing a decrease in the abundance and size of ostracods with increasing sediment age and depth. In addition, several PPPs detected in the ponds were found to correlate with ostracod fluxes, showing that the quality of recent sediment layers posed toxic risks to ostracods. The results also demonstrate that the sediments of the two studied ponds have been contaminated in the past (e.g., the 1960s) not only with PPPs but also with other toxic compounds such as trace elements (lead (Pb), copper (Cu), cadmium (Cd) and zinc (Zn)), and their high concentrations affected the abundance of ostracods in a greater level than PPPs.

This work contributes to the emerging field of paleotoxicology research in lakes and ponds and highlights the importance of interdisciplinary work to assess the fate and toxicity of anthropogenic compounds. Since ponds are biodiversity hotspots, this work highlights the urge and importance to address these valuable ecosystems in the context of anthropogenic pollution research and in policy decisions to improve their quality and ensure that these species-rich ecosystems persist in the future.

#### **2.06.P-We113 Effects of repeated pulsed deltamethrin exposures on a benthic community**

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The increasing use of pesticides in agriculture is one of the main reasons for the ecologically poor status of water bodies and especially of streams around the world. Pesticide inputs via surface runoff have been shown to be linked to rainfall events, resulting in high peak concentrations and complex exposure dynamics in streams that can have various effects on the aquatic environment. Based on available effect data, regulatory acceptable concentration (RAC) are derived for surface waters to avoid unacceptable effects on the environment. Several studies have shown, that pesticides may exceed their RAC value during rain events over a period of time. Current effect data of different pesticides is mostly based on single substance tests with single

target species conducted under laboratory conditions. However, in the aquatic environment, the biocoenosis is confronted with a variety of biotic and abiotic stress factors in addition to pesticide influx.

In an experimental approach using artificial indoor streams, a benthic community consisting of *Gammarus pulex*, *Chaetopteryx villosa*, *Lumbriculus variegatus*, and *Potamopyrgus antipodarum* was exposed to varying numbers of 12-hour deltamethrin pulses (one to four times) over a 35-day period with intervening recovery periods. The deltamethrin concentration for each pulse-like exposure was 0.64 ng/L (RAC value). At the end, various lethal and sublethal endpoints were determined to investigate whether there is a difference between a single exposure and up to four exposures, or whether the benthic community can recover after each pulse.

Preliminary results show that the ratio of survival and mortality of *C. villosa* within the treatment groups is significantly shifted after four pulsed exposures. In addition, the larvae of *C. villosa* exposed to deltamethrin tend to have lower energy levels. For *L. variegatus*, it was observed that the biomass tends to decrease with an increasing number of deltamethrin pulses. Our results indicate that unacceptable effects of multiple pulse-like deltamethrin exposures at concentrations equivalent to the RAC value on non-target aquatic invertebrates cannot be excluded. Moreover this experimental approach thus leads to more realistic effect data and can help to better assess the risks of pesticide exposure for aquatic organisms and to optimize regulatory risk assessment schemes.

#### **2.06.P-We114 Flushing away the future: The effects of wastewater treatment plants on aquatic invertebrates**

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Wastewater treatment plants (WWTP) are essential infrastructure in our developing world. However, with the invention and release of novel chemicals, in combination with frequent heavy rain events, most WWTPs fail to handle modern day tasks. Without upgrades, they are ineffective at fully removing micropollutants before treated effluents are released back into aquatic environments. Thus, WWTPs may represent additional point source impacts to freshwater environments, further pressuring aquatic communities. Studies – mostly focusing on single WWTPs – have shown that pollution tolerant taxa generally dominate impacted freshwater invertebrate communities. To expand on these findings, we analysed the effects of 170 WWTPs on invertebrate community composition. We selected suitable sampling sites upstream and downstream of the WWTPs and compared them using several diversity and pollution indices. In terms of abundance, the three most frequent and negatively impacted orders were the Plecoptera, Trichoptera and Gastropoda, while the Turbellaria, Hirudinea and Crustacea increased in abundance. Commonly used metrics showed no clear differences between upstream and downstream sites, however strong changes in community composition were observed (mean species turnover of 61%). This highlights the importance of analysing diversity from multiple perspectives, drawing right conclusions and assess ecological health adequately. Our results indicate that WWTPs change downstream conditions in favour of pollution tolerant taxa to the detriment of sensitive taxa. Responses on higher taxonomic levels can be informative, if analysed in combination with responses of lower taxonomic levels. Group responses can be driven by a few taxa, or opposing responses of species in the same group can result in an overall low order-level response. Upgrading WWTPs via additional treatment steps or merging might reduce adverse impacts, given upstream sections are in a good chemical and structural condition.

#### **2.06.P-We115 Decrypt the complex – Recent exposure influences neonicotinoid tolerance in a cryptic amphipod species complex**

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Cryptic species are rarely considered in ecotoxicology, resulting in misleading outcomes when using a single morphospecies that encompasses multiple cryptic species. This oversight contributes to the lack of reproducibility in ecotoxicological experiments and promotes false extrapolation of findings. The important question of ecological differentiation and the sensitivity of closely related cryptic species is rarely tackled, resulting in a substantial gap in our understanding of the vulnerability of individual species within a complex. Often non-target organisms such as amphipods are threatened by fertilizers and pesticides that are inserted in the river systems through run-offs. To better understand mechanisms influencing the tolerance of cryptic species complexes we tested the complex of the amphipod *Gammarus roeselii* against the neonicotinoid thiacloprid. The *G. roeselii* complex is a widely distributed amphipod all over Europe with a diversification hot spot in the Balkans. We sampled eleven populations in Greece and Germany belonging to four different MOTUs (Molecular Operational Taxonomic Units). Through COI barcoding we evaluated the MOTU status and calculated the genetic diversity for each population. We then tested the vulnerability to the neonicotinoid thiacloprid of each MOTU in a series of acute toxicity assays. Environmental and hydrological parameters as well as sediment in-vitro assays were used to evaluate the overall conditions of the sampling sites and a possible anthropogenic influence. With these parameters a PCA was carried out and the results of the EC<sub>50</sub> values (acute toxicity test) and genetic diversity were checked for correlation.

We found that the tolerance (EC<sub>50</sub> values) although MOTU-specific, are mainly and significantly different between the populations. The genetic diversity doesn't correlate with the tolerance. The first Principal Component of the environmental parameters tested correlates significantly negative with the EC<sub>50</sub> values.

These results indicate a strong influence of recent chemical exposure to the same or a similar pesticide rather than the influence of deep phylogenetic differences within the cryptic species complex. Our findings highlight the need to not only check the cryptic status of test organisms but also take the environment of each sampling site into account when analysing acute toxicity tests. These results show the importance of an integrative approach at analysing global stressors on aquatic systems.

## **2.06.PC New Developments in Sediment Ecotoxicology and Risk Assessment**

### **2.07 Pollinator Risk Assessment in a Changing Landscape**

#### **2.07.T-01 The Revised EFSA Bee Guidance (2023) One Year On: An Industry Stakeholder's Perspective and Recommendations**

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The European regulation of plant protection products (PPP) requires an evaluation of the risks to human health and the environment before the European Union level approval of active substances and national registration of formulated products (Reg (EU) 1107/2009).

In May 2023 the European Food Safety Authority (EFSA) published their revised bee pollinator guidance document. At the time of abstract submission (November 2023) this document is not adopted or implemented for use within the pesticide regulatory framework of the European Union.

In 2023 CropLife Europe (CLE) conducted an impact analysis for a range of uses of typical PPP across the European Union following the EFSA 2022 DRAFT bee Guidance document. Since the EFSA draft was published several changes have been made to the final document following a public commenting period. CLE has updated its analysis using the final inputs using the same toxicity and good agricultural practices (GAP) dataset as used in 2023. In addition to this, further analyses which were not included in 2023 have been conducted to investigate the performance of the proposed sublethal effects assessment and time reinforced toxicity (TRT) based on tier I assumptions.

This presentation reviews the performance of the new EFSA guidance proposal in the light of the outcome of an impact analysis performed for a range of PPP and uses, and case studies conducted by industry to test the useability and outcomes of applying the new draft proposal to real-life risk assessment situations.

It is recommended that the regulatory community proceed with caution with regards to a full and immediate implementation for regulatory purposes. Further development of approaches at lower tiers is still required and a road map is needed as not all aspects can be implemented, training is needed, and a working user-friendly calculator tool is required.

#### **2.07.T-02 A National Authorities View on the Revised EFSA Bee Guidance Document**

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The indisputably high economic and ecologic importance of honey bees justifies the necessity of a high level of protection of bees and bee colonies. Plant protection products (PPPs) are subject to a risk assessment.

In order to achieve the desired level of protection for honey bees in the regulatory context, numerous global efforts are underway to constantly improve testing methods and to refine risk assessment methodologies and risk assessment schemes on the basis of scientific knowledge but also to refine possible risk mitigation option. All risk assessment schemes rely on data generated at the laboratory, semi-field or field-level on the basis of reliable, if possible test methods that are validated as OECD guidelines. As a fundamental principle in different risk assessment schemes, a tiered approach is proposed, with laboratory tests in the first tier as a starting point, moving on to higher tier tests if the result of the first tier suggests that a risk cannot be excluded.

In Europe, revised EFSA guidance document on the risk assessment of plant protection products on bees published in 2023, defines the specific protection goal (SPG) for honey bees (*Apis mellifera*) to not exceed a colony size reduction of 10 % following exposure to plant protection products which contains numerous changes in approaches, calculations and assessments, as well as statistical calculations. In the talk, the view of a national risk assessment authority, the Julius-Kühn Institute will discuss the applicability, feasibility and potential areas of future improvements.

#### **2.07.T-03 Interpreting Acute Oral, Acute Contact, Glass Plate And Chronic Tests Within One Single Framework**

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Recently, it was shown that acute oral, acute contact and chronic tests for bees can be interpreted within one consistent framework, with one set of parameters: the BeeGUTS framework. Since all tests can be interpreted within one consistent framework results from one test can be extrapolated to any other test.

What is missing in this framework are indirect exposure tests. In these tests organisms are put on treated glass plates (or leaves) and take up the compound of interest through their legs and/or cuticle, which can generate an effect. In an indirect exposure test, the exposure concentration is expressed in g/surface area of the treated medium (glass plate or leave) and the actual dose is unknown. This hampers the interpretation and the extrapolation potential of such test as the relation between the exposure and the dose is unknown. This research aimed at including the indirect exposure test in the BeeGUTS framework, which would allow to also extrapolate results from the acute oral, acute contact or chronic tests to the indirect exposure test.

An indirect exposure test with bees exposed to Imidacloprid was carried out at the WENR laboratories and was used to translate the exposure from ng/cm<sup>2</sup> sprayed surface to an actual dose (in ng/bee) by using the BeeGUTS framework with known parameter values for Imidacloprid. Here the model was used to calculate the exposure from observed effects using known parameter values instead of calculating the parameters from observed effects and a known exposure. The assumptions that were made is 1) that toxicity is a combination of dose, kinetics and intrinsic sensitivity of an organism, independent of the test and 2) the concentration in a glass plate test is constant over time and the dose is linear with the exposure concentration in g/surface area.

These assumptions gave an almost perfect prediction of the effects in the indirect exposure test using the parameters of the chronic test as a starting point. This implies that we can now do one single test and predict the outcome of all other tests for Imidacloprid, but the BeeGUST framework showed that the general principles apply to the majority of tested compounds.

#### **2.07.T-04 Pesticide use negatively affects bumble bees across European landscapes**

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While we know some pesticides negatively affect bees, the scale of these effects beyond single substances in focal fields has remained unknown. Our work, funded by the European Commission, answered recent calls for a more realistic assessment of the risks posed by mixtures of commonly used pesticides at landscape scales. We conducted a field-based study spanning 106 sites across eight European countries, deploying 316 *Bombus terrestris* colonies. We show that the multiple pesticides found in bumble bee-collected pollen are associated with reduced colony performance (fewer offspring) during crop bloom, especially in simplified landscapes with intensive agricultural practices. These findings confirm that the regulatory system fails to sufficiently prevent pesticide-related impacts on non-target organisms, even for a eusocial pollinator species where colony size may buffer against such impacts. Furthermore, our findings support the need for post-approval monitoring of both pesticide exposure and effects to confirm that the regulatory process is sufficiently protective in limiting the collateral environmental damage of agricultural pesticide use.

#### **2.07.T-05 Ecological Effect Models for Bee Risk Assessments**

Amelie Schmolke, Matthias Becher, Wally Bluhm, Oliver Jakoby and Johannes Lückmann, RIFCON GmbH, Germany

Ecological effect models were identified as valuable tools for the evaluation of possible risks during the registration of plant protection products in Europe. With the publication of the revised EFSA Bee Guidance Document (EFSA 2023), such tools become increasingly important for the higher-tier risk assessment of bees which focuses on potential effects on colonies and populations. Ecological effect models have the potential to provide important and reliable tools that can be used to increase the reliability of data generated from labor- and people-intensive field studies. In addition, the great diversity of bee species, their different levels of sociality and their ecological traits indicate that models are needed to extrapolate recorded effects of surrogate species. In the context of the risk assessment for bees exposed to plant protection products, colony and population models have already been developed. Model applications include, for instance, the extrapolation of exposure-effect responses from organism to colony or population level, the estimation of observed effects in field-based studies to other species or environmental conditions as well as the comparison of possible mitigation scenarios. We will present and discuss different models for honey bees, bumble bees and solitary bees that are currently available. We will provide an overview of the current state of the art and discuss how bee models can be used in combination with tailor-made field studies or field-based study data. Furthermore, we will also address model performance evaluation, which is identified as prerequisite for model acceptance by authorities.

#### **2.07.P Pollinator Risk Assessment in a Changing Landscape**

#### **2.07.P-Tu147 The Revised EFSA Bee Guidance – First Hands-on Experiences In Risk Assessment And Lessons Learned So Far**

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We reviewed the European Food Safety Authorisation (EFSA) revised Pollinator guidance document published in 2023 with the aim of supporting future development of the GB regulatory framework for pollinators post-EU exit. Our objectives were to provide a road map for identifying best practice by summarising the uncertainties, data gaps, data limitations and research gaps in current regulatory risk assessment frameworks. We found that the currently proposed approaches still fail to adequately account for differences in key biological traits that may influence the sensitivity and exposure of the c. 260 UK bee pollinator species to plant protection products. In particular, understanding exposure risk for ground nesting bees is limited. Development of ground nesting species (e.g., *Andrena*) as models for higher tier studies to compliment the use of the cavity nesting *Osmia bicornis* would be a major step forward. The absence of large, replicated, field trials that are realistic in terms of cropping area, landscapes and agronomic practice, along with failure to capture and predict synergistic interactions or factors that affect sensitivity to pesticides (e.g., disease) continues to hinder progress around designing effective higher tier assessments. A lack of linkages between individual-level effects and colony- or population-level effects for honey bees, bumble bees and wild bee species has made it difficult to define specific protection goals. But, with models like BEEHAVE, steps towards achieving this are now much closer to being operational. Post-approval monitoring of a broader range of bee species would greatly improve our capacity to support longer-term assessments of hazard or risk that could not realistically be achieved via standard pre-approval risk assessment protocols. Post-approval monitoring would also provide an opportunity to improve our understanding of emerging risks under field conditions, the scale over which pollinators forage on blocked treated crops, and synergistic effects. We highlight the need of a framework for reactive implementation of mitigation measures to help protect bees and maintain viability of plant protection products.

### 2.07.P-Tu148 The Natural Variability of Honey Bee Colonies - How to Meet the Requirements of the New EFSA Bee Guidance

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The revised EFSA guidance document on the risk assessment of plant protection products on bees (2023) defines the specific protection goal (SPG) for honey bees (*Apis mellifera*) to not exceed a colony size reduction of 10 % following exposure to plant protection products. For bumble bees and solitary bees no threshold could yet be defined due to a lack of data.

The natural variability of honey bee colonies ranged between 10 % and 53 % around the mean of the initial colony strength in five different large-scale field studies that included 18 to 48 control colonies. Despite this relatively large number of colonies, four out of five studies would not have enough replication and would likely fail the equivalence test under the new requirements of the EFSA bee guidance. Under realistic conditions, a 10 % reduction in the colony size will be hardly detectable when the natural variability is high among honey bee colonies. Several measures are proposed to reduce the natural variability, e.g. increase the number of colonies in the study, use colonies derived from sister queens and select those from an excess of colonies that are most similar in colony strength, to name only some. The revised EFSA bee guidance also suggests adding experimental colony data from the following year, if the number of replicates was insufficient to achieve adequate power in the equivalence test, for example, due to high variability in the first study year. However, these proposals could increase the variability among the colonies. Increasing the number of replicates would also potentiate the time for colony assessments. These should ideally be done by one person to minimize the estimation error, but even a skilled assessor can handle hardly more than 36 colonies per day. Hence, different environmental conditions and continued colony development over the days of the assessments would add variability to the data. Also, the required spatial extent of the study leads to different landscape characteristics, causing differences in weather conditions and food supply among the colonies.

### 2.07.P-Tu149 Does the distinction among honeybee subspecies matter for pesticide risk assessment?

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The recent framework devised by the European Food Safety Authority (EFSA) reports a lack of research data on potential differences in the toxicological sensitivity among honeybee (HB) subspecies. *Apis mellifera* is recognized by the Organisation for Economic Co-operation and Development (OECD) as a model organism for risk assessment (RA) studies. However, it shows considerable variation, as reflected by at least 24 subspecies. We therefore used the mortality to investigate and to compare the responses among three European subspecies exposed to pesticides. Newly emerged bees from three HB subspecies (*A. m. iberiensis* - AMI, *A. m. ligustica* - AML, and *A. m. carnica* - AMC) were subjected to a unique concentration of each pesticide (tau-fluvalinate, TAU and flupyradifurone, FPF). The trials for all the 3 subspecies were carried out at the same period, under equal conditions, and in accordance with the OECD (guideline 245). There is clear evidence that the three subspecies responded distinctly to the pesticides. Mostly, AMI was the most affected subspecies, showing the highest mortality rates. This evidence contradicted our expectations since AMI presents a more defensive behavior than the other two subspecies. By using a primary endpoint posed by the OECD (mortality), our findings provide essential

insights for further research into this topic. We are currently investigating a set of sublethal effects at molecular level. Our outstanding results will directly contribute to four of the nine knowledge gaps outlined by the EFSA's framework. We will address it further for the EFSA risk assessment proposals. Our study represents the first comparative approach of responses to pesticides among three honeybee subspecies. We have brought a new approach that is up to date overlooked in risk assessment studies.

#### **2.07.P-Tu150 From Individual to Colony Level Effects: How Should Colony Structure and Timescales be Considered?**

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In May 2023 European Food Safety Authority published their revised revised Pollinator guidance document. At the time of abstract submission (November 2023) this document is not adopted or implemented for use within the pesticide regulatory frame of the European Union. For screening and tier I risk assessments the revised scheme does not propose to use trigger values but directly compares the outcome of the risk calculation to a specific protection goal (SPG) using a combined effect approach. For honey bees it is proposed that this can be achieved in 3 steps; 1) quantification of effects at individual levels, 2) extrapolation of the individual level effects to colony and 3) combination of effects at the colony level.

The extrapolation step 2 assumes a conservative 1:1 propagation of individual to colony level effects for all experiments, i.e., using dietary and contact exposure. Multiple concerns have been raised about this approach as it does not consider several factors such as the population structure (e.g., pupal bees are not being fed so there is no dietary exposure and the different time scales of acute and chronic effects). In this paper we investigate the level of conservatism based on this assumption and suggest alternatives that could be used as a tier 2 refinement before directly generating higher tier effect studies. These approaches include: i) consideration of colony structure, ii) use of TK/TD models (e.g., BeeGUTS), iii) application of colony level population models (e.g., BEEHAVE), iv) use of colony level ecotoxicological models (e.g., BEEHAVE<sub>ecotox</sub>) and v) comparison with the outcomes of semi-field and field effects studies.

We make recommendations on how the extrapolation at step 2 can be achieved in a more realistic way and discuss future options such as implementation of a modeling framework and applicability to tier I and II risk assessments for bumblebee colonies and solitary bee populations.

#### **2.07.P-Tu151 Acute Toxicity of Dichlorvos and Carbendazim to *Apis mellifera* Larvae Reared In vitro**

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A wide variety of agricultural crops are pollinated by the honeybee (*Apis mellifera* L.). For foraging, the honeybee covers a distance of 3 - 8 km from the hive, sometimes even longer. Honeybee carries many of the toxic compounds that exist in its environment during foraging. Although honeybees are non-target organisms for most pesticide applications, they nevertheless are exposed to pesticides while collecting pollen, nectar, saps/resins, and water. Hence, larvae are exposed to pesticides in contaminated nectar, pollen and wax. The reported high loss rates of managed honeybee colonies have been attributed to diverse stressors including pesticides. It is more common to check the pesticide toxicity on adult worker honeybee than that on larva due to challenge in rearing larvae *in vitro*. Herein, we aimed to assess the acute toxicity of an organophosphate insecticide, Dichlorvos 76% EC (DCL) and a systemic fungicide, Carbendazim 50% WP (CBD) to *Apis mellifera* larvae reared *in vitro*. In our recent study, we exposed honeybee larvae (reared *in vitro*) to DCL and CBD according to OECD Test Guideline 237. DCL and CBD were exposed to honeybee larvae at a range of 0.005 to 3.8 µg/larva and 0.3 to 200.0 µg/larva, respectively. The median lethal dose (LD<sub>50</sub>) of DCL and CBD was calculated for larva at 72 h following single diet exposure. The LD<sub>50</sub> value for DCL was 0.121 (0.061 – 0.242) µg/larva. While there was no effect of CBD on larvae, the LD<sub>50</sub> value was greater than 200.0 µg/larva (100 µg a.i./larva). These findings reflect an evaluation of effects of DCL and CBD on honeybee larvae. There is also further scope to check long-term toxicity of developing honeybees after repeated exposure. Overall, our research is useful for determining acute toxicity of pesticide to *Apis mellifera* Larvae.

#### **2.07.P-Tu152 Testing the Flight Ability of Honeybees to Return to the Hive After a Single Oral Exposure to Sublethal Doses of Deltamethrin**

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Plant protection products (PPPs) are only allowed on the market after a thorough environmental risk assessment. To carry out the assessment, it is necessary to perform a number of ecotoxicological tests on non-target organisms, including the honeybee (*Apis mellifera* L.). It is suspected that the chemicals used may disturb bees' physiological functions and orientation in the field, which are important when flying in search of bee forage. The consequence of this phenomenon is shortening the distance of beneficial flights and reducing the number of insects returning with food. This leads to weakening of the bee colony and its increased susceptibility to diseases. From the point of view of apiary management, the efficiency of honey production decreases and financial expenditure on maintaining the apiary increases. Due to the increasing environmental pollution with plant protection products and their residues, the package of tests required for registration of plant protection products is gradually expanding. In July 2021, the Organization for Economic Co-operation and Development (OECD) introduced Guideline No. 332, which covers the study of the impact of sublethal doses of chemical substances and their mixtures on the effectiveness of return flights of honeybees (*A. mellifera* L.). One of the chemicals actively used in agriculture is deltamethrin.

It is an active substance with highly effective insecticidal effect. An organic chemical compound from the group of synthetic pyrethroids. It is a neurotoxin intended to combat pests with biting and sucking mouthparts in vegetable, fruit and ornamental crops, as well as to protect grain. Although it should be applied outside the period of honeybee activity, these insects may be exposed to residues found in the environment.

A semi-field study was carried out, in which bees were marked and exposed to a number of sublethal doses of deltamethrin (contained in the diet) in the laboratory, then the bees were released in the field (1 km from the apiary), and their return to the hive was recorded. The number of returns of foragers exposed to the tested chemical and the unexposed control group was compared. The monitoring period lasted 24 hours from the moment the bees were released in the field. The comparison was possible by monitoring experimental bees using radio frequency identification (RFID) tagging technology.

#### **2.07.P-Tu153 Weeds in Cropped Fields – Effects on Honeybee Colony Development Using BEEHAVEecotox**

*Matthias Becher, Johannes Lückmann, Wally Bluhm, Marcus Metz, Amelie Schmolke and Oliver Jakoby, RIFCON GmbH, Germany*

Weeds in treated fields can potentially be one exposure route for foraging honeybees. Whereas the first EFSA Bee Guidance (2013) regarded this scenario relevant if at least 10% surface area of a treated field is covered by attractive weeds at the time of application, the revised bee guidance document (EFSA 2023) considers this scenario relevant depending on the crop and the timing of application. In either case, lower tier risk assessments often indicate a potential risk for honeybees from weeds in treated fields. However, the relevance of this scenario as a potential threat for a colony and its strength is unclear and depends on a number of factors.

While empirical data or studies on this subject are very limited, computer simulations can be a very useful and cost-effective way to shed some light on this complex issue. Therefore, we used the recently developed ecotox module BEEHAVEecotox of the honeybee model BEEHAVE to improve our understanding of potential effects of weeds on honeybee colony development in the light of the upcoming bee risk assessment. BEEHAVE is an established, agent-based computer model that has been evaluated and used by EFSA, researchers, and industry. It simulates foraging behaviour in a realistic landscape and colony development in detail.

We analysed different simplified scenarios with colonies placed close to arable fields, which are either free of weeds or covered by a certain proportion of weeds. The attractiveness of these weeds was considered higher or lower in comparison to the crop. Furthermore, the weeds were either assumed in flower throughout the year or only during the application period of a pesticide. In addition, the simulations included varying toxicity of the applied pesticide.

The obtained results emphasise the trade-off between risk to pesticide exposure and the additional resources provided to bees. They are presented and discussed in the context of the the honeybee risk assessment.

#### **2.07.P-Tu154 Using the BEEHAVE Honey Bee Model Across Climates: Queen Egg-Laying Emerging From Weather, Pollen Storage and Brood Size Rather Than Being Imposed**

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Multiple stressors increase the risk of honey bee colony collapse and winter mortality. Empirical studies to determine the relative importance of stressors and critical stress levels are not feasible because most stressors and colony characteristics cannot be controlled. Modelling of honey bee colonies can help to fill this gap. The BEEHAVE model takes into account local land use, including pesticide use, weather, mite infestation and beekeeping practices, and has been used in more than 20 studies. However, it is based on an imposed egg laying rate which is only representative of average central European conditions. We developed a modified version, BEEHAVE-PPE (Pollen Pheromone Egg-laying), where the maximum egg laying rate depends on pollen storage. We assumed that daily pollen foraging, and thus pollen uptake, depends not only on the weather, but also on brood size, brood pheromones, and temperature. BEEHAVE-PPE was successfully calibrated using demographic and weather data from the ECOBEE study in France. We then applied the calibrated model to three sites across Europe (Spain, Romania, Ireland) where a previous study commissioned by the European Food Safety Authority (EFSA) had used the original BEEHAVE model, but with the imposed egg-laying and resource availability shifted along the time axis. The EFSA simulations were not validated, for the lack of data, but they were indirectly checked for consistency. The BEEHAVE-PPE results captured some aspects of the EFSA simulations, but differed in others and were overall more variable between sites. In conclusion, our results indicate that it is possible to let the egg-laying rate in honey bee colonies emerge from the local climate and the effects of brood pheromones. Although our new version of BEEHAVE is too experimental to be used in applications, it suggests that it would be worthwhile to experimentally study the effects of brood pheromones, temperature and other factors on pollen foraging and, in turn, the queen's egg-laying.

#### **2.07.P-Tu155 A TKTD module for BEEHAVEecotox – combining BEEHAVEecotox and BeeGUTS**

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Honey bees provide the important ecosystem service of pollination to wild plant species and cultivated crops. Thus, they are a

crucial part of the environmental risk assessment of plant protection products in the European Union. In this context, mechanistic modeling offers a powerful tool to predict the exposure and effects on bees in the field and find increasing acceptance within the regulatory framework.

The BEEHAVE<sub>ecotox</sub> model is the first honey bee model to mechanistically link the realistic exposure in the field with subsequent effects on different levels of the bee colony. It uses standard regulatory study data to derive dose-response functions of effects resulting from contact and oral exposure and also considers larval mortality.

In parallel, the BeeGUTS model was developed, which is a TKTD model for the interpretation and extrapolation of honey bee survival data. The model provides one consistent framework integrating the effects from acute contact, acute oral, and chronic oral studies into one set of parameters by considering toxicity as a process in time. This new integrative approach moving from single point estimates of toxicity and exposure to a holistic link between exposure and effect.

Both models have been validated with independent datasets and provided good predictions of honey bee effects within their domain of applicability. Since the BEEHAVE<sub>ecotox</sub> model was developed with a modular modelling approach in mind, it allows to exchange or add modules when new knowledge is available.

In this poster we will present the first results of the integration of a new toxicity module into BEEHAVE<sub>ecotox</sub> using BeeGUTS. The poster will highlight the challenges and differences between the two approaches and compare predicted results of semi-field studies.

### **2.07.P-Tu156 Predicting environmental pollution status and pollen diversity at the pan-European scale using citizen science monitoring data**

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The natural environment is under pressure as a result of human activities. In order to survey and monitor landscape quality, there is a need for a general approach that allows for an integrated assessment of the contamination and habitat quality at large spatial scales and over the course of a season. The INSIGNIA-EU project aims to address this need. The honey bee (*Apis mellifera* L.), an important pollinator species, is used as a biomonitoring tool, utilising the central-place foraging behaviour of honey bees to collect information on the environmental quality of the surrounding area.

Data on pollen, pesticides, VOC/PAHs, heavy metals, and microplastic were collected by 315 Citizen scientists (CS) in the 27 EU countries in 2023. Sites were chosen to cover different landscapes based on CORINE landscape classes such that an equal spread in space, land-use classes, and diversity was achieved. Clustering of the 315 CS locations was performed to determine similarity of the surrounding landscape and to define a sampling frame. Subsequently, grid cells were compared against clusters to filter out grid cells that were outside of the sampling frame. Prediction of the probability of a pollen family or a pollutant being present in a location (i.e., multi-class classification) was achieved using random forest models. Separate models were created for pollen and the different types of pollutant.

The distribution of apiaries across land-use classes and the diversity was not even, this can be explained by the fact that, for example, beekeepers do not tend to keep bees in heavily forested areas for a lack of foraging resources. Consequently, monitoring and modelling results are context dependant. Large-scale differences can be seen, as well as local changes over the course of a growing season for both pollen and pollutants.

Our approach linked land-use to the pollen and pesticide data generated during the INSIGNIA EU to create predictive maps of: 1) pollen diversity, and 2) pollutants. This study represents an unprecedentedly large and comprehensive dataset on the status of environmental pollution and pollen diversity. The maps created in this study can be used by, for example, beekeepers to find suitable locations for their apiaries. Moreover, these maps will show, in time and space, where mitigation measures may be needed for different types of pollution, allowing authorities and managers to take efficient and targeted action.

### **2.07.P-Tu157 SolBeePop-ecotox: a population model for higher-tier risk assessments of solitary bees**

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Solitary bees are considered in pesticide risk assessments of pollinators and explicitly addressed in the new guidance on bees by EFSA (2023). In agricultural landscapes, solitary bees occur in a large diversity of species. A few species are managed specifically for crop pollination. Their solitary life style distinguishes them from honey bees and bumble bees, and their ecology can result in differences in exposures to pesticides as well as long-term outcomes on the population level. Studies have been conducted to assess risks to solitary bee populations from pesticide exposure via treated fields. However, such studies are time- and resource intensive and focus on the combination of a single species, crop and year. With the population model for solitary bees, SolBeePop<sub>ecotox</sub>, we demonstrate the application of a model that can be applied in to expand the use of study data. Available data from semi-field studies with the red mason bee, *Osmia bicornis*, were used to assess the model performance, simulating bees foraging in tunnels over control and insecticide-treated oilseed rape fields. Effects are implemented in the model using a simplified toxicokinetic-toxicodynamic model, BeeGUTS, adapted specifically for bees. We applied the model to simulate hypothetical semi-field studies with additional solitary bees species, including a cavity-nesting species, the alfalfa leafcutter bee, *Megachile rotundata*, and two soil-nesting species, the alkali bee, *Nomia melanderi*, and the hoary squash bee, *Eucera pruinosa*. The model application demonstrates how models can inform higher-tier risk assessments, expanding available empirical data to untested conditions and become important tools to support pesticide risk assessments.

#### **2.07.P-Tu158 Risk assessment of bees from the use of biocide – exposure assessment**

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During 2019, ECHA received a mandate from the European Commission to develop a guidance for assessing the risk to pollinators (including bees) from the exposure to biocides. According to the mandate, ECHA should take into account the revised EFSA Guidance document on the risk assessment of plant protection products on bees, which was released in May 2023.

In 2023, ECHA has finalised a first version of the guidance document (GD) which provides a methodology to assess the risk to bees that are exposed to biocides. This is done by following a tiered approach for the exposure and the effect assessment. In the risk assessment of honey bees, the magnitude dimension of the Specific Protection Goals is applied as a threshold for acceptable effects. The GD proposes a quantitative risk assessment approach to assess the risk to bees from the biocidal product type (PT) 18 (insecticides, acaricides and products to control other arthropods) emission scenarios. The possible sources of exposure covered in the GD are (1) large scale spraying, (2) application of manure/sludge to agricultural soil or grassland, (3) small scale spray around the house, and (4) irrigation of private gardens with treated water. While the focus in the first version of the ECHA guidance is on PT18 active substances, there may be instances where a PT18 active substance in a product in another PT also warrants an assessment when the exposure is considered significant enough to warrant further consideration.

The aim of this presentation will be to provide the audience with an overview of the methodology to assess the risk to bees that are exposed to biocides. In particular, the relevant sources of exposure from the use of biocides will be described. The tiered approach starting from screening step and proceeding to Tier, Tier 2 and Tier 3 will be explained. This method in the new ECHA Bee guidance will provide a quantitative approach for the exposure assessment of bees that has been lacking in the previous ECHA guidance documents for the risk assessment of biocides.

#### **2.07.P-Tu159 Biocides and Pesticides perspective on the risk assessment of pollinators: ECHA and EFSA Bee guidance and developments for other pollinators**

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In March 2019, EFSA received a mandate from the European Commission to revise the 2013 bee guidance document for plant protection products (PPPs). As a crucial aspect of the revision, EFSA was requested to consider the EU Commission activities on the revision of the specific protection goal.

In December 2019, the European Commission mandated ECHA to develop a guidance for assessing the risks to arthropod pollinators (including bees) from biocides exposure. Although ECHA and EFSA developed their guidance documents (GDs) independently, the two GDs were aligned as far as possible, in relation to respective legal frameworks for pesticides and biocides.

The revised EFSA guidance on the risk assessment of plant protection products on bees was published in May 2023. A first version of the ECHA Guidance on the assessment of risks to bees from the use of biocides was published in April 2023, and a final version is expected by end of March 2024. Regarding non-target arthropod pollinators other than bees, in 2022 ECHA published a scientific report on European arthropods and their role in pollination.

The aim of this presentation is to provide the audience with an overview on the new developments of guidance in the area of risk assessment of pollinators from the exposure to biocides and PPPs. The development process, scope and biocide-specific aspects regarding risk assessment of bees different from PPP assessment will be described. The commonalities and differences between the two GDs for the risk assessment of bees will be highlighted, as well as the identified research needs.

In addition, the state of play with regards to knowledge on non-bee pollinators and future needs for guidance development will be presented.

#### **2.07.P-Tu160 Investigation of Key Parameters to Adapt Ecotoxicological Testing to Microbial Pesticides – An Example With a Social Insect and an Aquatic Invertebrate**

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There is a growing interest in developing biopesticides as sustainable tools in the global plant protection market. Biopesticides are naturally occurring substances that control pests by biological mechanisms. In contrast to synthetic chemicals, microbial pesticides are relatively specific and have targeted modes of action that include, resources competition, infectivity, and pathogenicity, rather than toxicity alone. Such mechanisms present potential ecological risks to non-target organisms and must be assessed considering concrete environmental scenarios (e.g. exposure routes, development, and life cycles). Current testing guidelines adapted to chemicals toxicity must be adjusted according to the ecological characteristics of the tested organisms, to properly assess the associated risks. Here, we evaluated the effect of a widely used microbial pesticide on a non-target social insect and an aquatic invertebrate by adapting different parameters of the study protocols to better mimic the reality of a microbial exposure. Honey bee larvae and young daphnids were exposed to the bacterium *Bacillus thuringiensis* and the effect on their survival and development was assessed. A microbiology-based method was used to verify the bacterial dose administered to both test systems over the exposure period. The testing parameters were successfully adapted to evaluate the effect of *B. thuringiensis* on the development of honey bee larvae and young daphnids. The challenges and opportunities associated with the ecological interactions (e.g. exposure routes) in ecotoxicological testing were highlighted and discussed. These investigations are essential to ensure a correct ecological risk assessment of emerging microbial control agents for non-target organisms.

#### **2.07.P-Tu161 Oral exposure-driven effects of a novel spider venom-based biopesticide on survival, gut microbiome and head transcriptome of adult worker honeybees**

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The escalating use of plant protection products in recent decades contributed to the degradation of numerous ecosystem services by exerting heightened pressure on non-target organisms (NTOs), many of which play crucial roles as service providers. To overcome these issues, the European Union through the Farm to Fork Strategy and its aim of reducing the use of chemical pesticides by 50% and the use of more hazardous pesticides by 50% by 2030, targeted the development of novel and more environmentally friendly products (the “low-risk pesticides”). Biopesticides derived from natural molecules of animals, plants, bacteria and minerals are considered a promising alternative to conventional chemical pesticides. One source of molecules currently receiving attention are proteins from insect antagonists, such as the neurotoxin from the venom of the spider *Segestria florentina* (SF16), which when fused to bovine serum albumin (BSA) proved to be toxic to different insect pests of important crops. Although biopesticides are often considered to have a reduced environmental impact, their effects on NTOs can be challenging to predict, mainly because of the lack of appropriate tools incorporated into the regulatory framework. The study aimed to determine the effects of the fusion protein SF16-BSA upon oral exposure on *A. mellifera*, the key model organism for pesticide Environmental Risk Assessment to pollinators. The effects were evaluated, after acute and chronic exposure, on the survival, gut microbiome and head transcriptome of adult worker honeybees. Acute and chronic oral toxicity tests were performed according to the Organization for Economic Cooperation and Development Guidelines for the Testing of Chemicals - Tests No. 213 and 245, respectively. DNA (gut) and RNA (head) extractions were made using NZY Isolation kits (NZYTech), following the manufacturer protocols. Sequencing was made using an Illumina NovaSeq platform at Novogene facilities (United Kingdom). We demonstrated that acute oral exposure to SF16-BSA had no adverse effects on honeybees. On the other hand, when bees were subjected to chronic exposure, it caused no significant effects on survival but induced changes in the gut microbiome and alteration in gene expression profiles. The responses revealed by the “omic” approaches, coupled with regular testing procedures could be considered valuable tools for further research and inclusion in regulatory frameworks.

#### **2.07.P-Tu162 Beyond Managed Bees: Understanding Non-Target Arthropods for Enhanced Pollination Services in EU Agricultural Systems**

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Pollination, an essential ecosystem service (ES), plays a vital role in sustaining life on Earth, especially for humans. However, the current emphasis on a limited set of non-target arthropods (NTAs) in risk assessment neglects the broader spectrum of species. EU Member States express concerns about the inadequacies of existing methodologies and the ambiguity surrounding the level of protection afforded. Recognizing representative NTAs beyond managed bees, which drive ES delivery, becomes crucial for targeted risk assessment and ecological relevance. This study aims to identify and compare the distribution of NTAs

in EU agriculture, analysing variations across different crops and EU geographical zones. The study concentrated on six annual crops (wheat, maize, barley, oilseed rape, potato, beet) and three perennial crops (apple, grape, olives) chosen for their significant contribution to EU production and data availability. Arthropod data for arable crops was sourced from an existing database [1] and a literature review on perennial crops was conducted using CAB Abstracts. The EU-focused invertebrate dataset, excluding pest species, underwent non-metric multidimensional scaling analysis to compare NTAs compositions. Representative NTA families were identified based on criteria like abundance, dominance, diversity, and distribution. Surprisingly analysis reveals that, among perennial crops, only olives and grapes exhibit close grouping. Wheat appears closely associated with oilseed rape and potatoes, despite the latter two being flowering crops. Unexpectedly, the separation by crop and EU zones is not straightforward. While olives and grapes cluster, apples in all three zones seem more like other crops from the central zone. Identified key NTAs families include *Andrenidae* (mining bees), *Apidae* and *Halictidae* (bees), *Noctuidae* (owlet moths), and *Syrphidae* (hoverflies). The recognized families, extending beyond conventional test species, highlight the imperative for more precise testing strategies oriented towards ES to enhance guidance in risk assessment within agricultural ecosystems. Specifically, because they represent a wide range of traits and may have different sensitivities and handle exposure and recovery differently. The findings underscore the necessity for a more precise and ecosystem-service-oriented approach in developing risk assessment guidance for pesticide use in agriculture.

[1] Riedel et al. 2016. EU. EFSA Supporting Publications, 13, 956E.

### **2.07.P-Tu163 Chronic Bumblebee Feeding Test under Natural Environmental Conditions – Lessons Learned so Far** *Annika Alscher<sup>1</sup>, Johannes Lückmann<sup>1</sup>, Nadja Schnetzer<sup>1</sup>, Lea Franke<sup>2</sup>, Julian Fricke<sup>2</sup> and Olaf Klein<sup>2</sup>, (1)RIFCON GmbH, Germany, (2)Eurofins Agroscience Services Ecotox GmbH, Germany*

According to the revised EFSA Bee Guidance (EFSA 2023) semi-field and field testing on bumblebees is proposed to gain insight into the risks for bumblebee colonies after pesticide application to flowering crops. If the lower tier risk assessment shows a strong dominance of the dietary exposure route, it is recommended conducting feeding tests with a two-week exposure period under field conditions without having agreed study protocols in place. Annex C of the revised guidance presents a rather rough methodological description that lacks detailed information about feasibility of such tests and the generation of comparable and reproducible data. In particular, the proposed test method includes a positive control, but does not define a specific substance or feeding concentrations for the test organisms.

We conducted feeding tests with free flying bumblebee colonies under natural environmental conditions, and at two different sites in Germany. The commercially available small colonies were placed randomly along a linear structure in the landscape with 10 m distance between single colonies to reduce risk of worker drift between colonies of different treatment groups. Colonies were offered a spiked sugar solution via a feeder installed beneath the nest inside the nest box over a period of two weeks and were monitored the following six weeks. During study duration bumblebees could forage for pollen and nectar. Endpoints assessed were colony weight as a proxy for colony strength as a specific protection goal of EFSA, mortality and queen production.

Dimethoate EC 400 (active substance dimethoate) and Dimilin 25 W (active substance diflubenzuron) were tested with a range of concentrations as positive controls. Dimethoate is regularly used for higher tier testing as a positive control in pollinator studies and acts on adult mortality whereas diflubenzuron is known to impacts larval development. As a negative control, colonies were fed with untreated sugar solution. The obtained results are presented and discussed.

### **2.07.P-Tu164 Non-Apis Bee Risk Assessment in the Absence of a Specific Protection Goal: Decision-Making With Limited Options**

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Recently, EFSA published their “revised guidance on the risk assessment of plant protection products on bees”. In addition to honeybees, the guidance requires the risk assessment for two additional bee groups: bumble bees and solitary bees. Unlike for honeybees no specific protection goal was defined for these groups. According to the European Commission the risk assessment would therefore require field studies with these bee groups as default. Given the lack of lower tier risk assessment, several risk management strategies have been proposed. Field studies may not be necessary if lower tier risk assessments for honeybees and non-target arthropods show no effects, which raises several follow-up questions such as the exact definition of “lower tier” or “no effects”. Other risk management proposals include laboratory studies with surrogate species from the respective bee group in combination with toxicity thresholds (LD<sub>50</sub> of > 100 µg/bee considered as non-toxic) or semi-field testing with surrogate species. Here, we investigated the feasibility of these proposals with regards to data and method availability and compared the outcome of different risk management strategies to the outcome of the honeybee risk assessment for a substantial number of compounds. The analysis aims to provide an overview about several risk management strategies for non-*Apis* bees in the absence of specific protection goals and will help to inform stakeholders about the practicability of EFSA’s revised guidance for non-*Apis* bees.

### **2.07.P-Tu165 Evaluation of the oral toxicity of the fungicide difenoconazole on native bees of the species *Scaptotrigona postica* and its subeffects.**

**Gabriela Reani Rodrigues Garcia<sup>1</sup>, Rafaela Garcia da Silva<sup>2</sup>, Joyce Oliveira Costa<sup>1</sup>, Annielly Fernanda de Sousa Silva<sup>2</sup>, Janete Brigante<sup>1</sup>, Eny Maria Vieira<sup>3</sup> and Michiel Daam<sup>4</sup>, (1)University of São Paulo (USP), Brazil, (2)Dqfm, USP, Brazil, (3)DQFM, University of São Paulo (USP), Brazil, (4)CENSE, New University of Lisbon**

Bees are insects of great importance for biodiversity and agricultural production, as they are responsible for promoting pollination, fruit generation, and providing honey, wax, propolis, and pollen. In Brazil, there is a wide variety of native bees known for not having stings or venom. Focusing on the bee, popularly known as Mandaguari (*Scaptotrigona postica*), its role as a pollinator is highly efficient, as the number of individuals per hive can reach more than 20,000 bees, positively impacting the pollination process. This study aimed to determine and compare the lethal concentration of the analytical standard difenoconazole and the commercial product SCORE (LC<sub>50</sub>), as well as their lethal dose (LD<sub>50</sub>), for the species of bee *Scaptotrigona postica*. Additionally, the study aimed to analyze the protein profile of Mandaguari in response to exposure to this fungicide using the biochemical test of polyacrylamide gel electrophoresis. For this study, foraging adult bees were collected in the early morning and brought to the laboratory. The bees were acclimated in a BOD incubator at a temperature of 26 ± 1°C, with food replenishment until the test. Bees were exposed to the following standard difenoconazole concentrations 20; 15; 12; 7; 2.5 and 1.5 mg mL<sup>-1</sup>, already for the commercial product, SCORE, the following concentrations were tested: 0.0065; 0.005; 0.0035; 0.025; 0.015; 0.0005 mg mL<sup>-1</sup>. The LC<sub>50</sub> was determined to be 19.99 g L<sup>-1</sup> (48h) and the LD<sub>50</sub> 0.095 mg a.i./bee (48h) for the difenoconazole standard, and for the commercial product SCORE, the LC<sub>50</sub> was 0.00682 g L<sup>-1</sup> (72h) and the LD<sub>50</sub> was 0.03136 µg a.i./bee (72h). It was observed that for the tested concentrations of both products, the higher concentrations showed mortality rates between 80 and 100%, as well as physical effects such as difficulty in flight and feeding. Finally, regarding the protein profile, an intensification of some enzymes, such as catalase and peroxidase, was observed in individuals exposed to difenoconazole, a strategy used by bee organisms for detoxification and tolerance.

### **2.07.P-Tu166 Toxicity of imidacloprid and thiamethoxam to *Scaptotrigona postica* bees**

**Joyce Oliveira Costa<sup>1</sup>, Annielly Fernanda de Sousa Silva<sup>2</sup>, Gabriela Reani Rodrigues Garcia<sup>1</sup>, Rafaela Garcia da Silva<sup>2</sup>, Janete Brigante<sup>1</sup>, Michiel Daam<sup>3</sup> and Eny Maria Vieira<sup>4</sup>, (1)University of São Paulo (USP), Brazil, (2)Dqfm, USP, Brazil, (3)CENSE, New University of Lisbon, (4)DQFM, University of São Paulo (USP), Brazil**

Native bees play a fundamental role in pollination, contributing to reproducing approximately 90% of flowering plant species and 80% of economically important crops. However, pesticide contamination threatens this species' survival, resulting in reduced biodiversity in ecosystems and food supply. Therefore, it was necessary to determine safe doses of pesticides, such as imidacloprid and thiamethoxam, to ensure that the bee's function and survival are not compromised. For this purpose, a preliminary oral bioassay was conducted, involving the collection of *Scaptotrigona postica* bees in the morning, between 07:00 and 08:00. Adult bees were collected at the hive entrance using a 250 mL plastic cage containing a 50% (w/v) sucrose solution. Five solutions with concentrations of 1000, 100, 10, 1, and 0.1 µg L<sup>-1</sup> of the standard for each pesticide were tested. The tests were conducted in triplicate, in addition to the control group, to obtain a concentration range where sample mortality varied between 10% and 90%. For each cage, 200 µL of the contaminated solution was offered, and deaths were recorded 6 hours after the test started and then at 24-hour intervals up to 96 hours. After the preliminary bioassays, the following concentrations were tested for imidacloprid 672, 599, 484, 325, and 240 µg L<sup>-1</sup> of the standard, obtaining LD<sub>50</sub> (72h) of 0.00228 µg imidacloprid per bee. For thiamethoxam, solutions with concentrations of 1654, 790, 546, 375, 246, 194, and 100 µg L<sup>-1</sup> of the thiamethoxam standard were tested. The LC<sub>50</sub> (24h) was 336.3 µg a.i. thiamethoxam L<sup>-1</sup>. The design of the definitive tests followed the same experimental procedure as the preliminary tests. The results of the bioassays indicate a mortality of up to 52% in samples contaminated with imidacloprid-containing solutions and 8 to 100% for thiamethoxam, indicating that the species *Scaptotrigona postica* is more susceptible to neonicotinoids compared to *Apis mellifera* used as a reference in exposure protocols.

### **2.07.P-Tu167 Toxicity of thiamethoxam insecticide on the stingless bee *Scaptotrigona postica* Latreille, 1807**

**Annielly Fernanda de Sousa Silva<sup>1</sup>, Joyce Oliveira Costa<sup>2</sup>, Gabriela Reani Rodrigues Garcia<sup>2</sup>, Rafaela Garcia da Silva<sup>1</sup>, Janete Brigante<sup>2</sup>, Michiel Daam<sup>3</sup> and Eny Maria Vieira<sup>4</sup>, (1)Dqfm, USP, Brazil, (2)University of São Paulo (USP), Brazil, (3)CENSE, New University of Lisbon, (4)DQFM, University of São Paulo (USP), Brazil**

Native bees play an essential role in pollination, contributing significantly to the reproduction of various crops, especially those of economic relevance. However, there is observed population decline in these insects, correlated with contamination by pesticides, primarily neonicotinoids. In order to assess whether the use of the commercial product thiamethoxam may be harmful to bees of the species *Scaptotrigona postica* L., toxicity bioassays were conducted in the laboratory. Adult bees were exposed to Actara® 250WG, which contains the active ingredient thiamethoxam, through two acute exposure routes: oral and contact. Acute toxicity bioassays were conducted following the OECD N° 213 and 214 protocol guidelines. Bee collection was performed between 7:00 and 8:00 a.m., directly at the hive entrance using a 250 mL plastic cage containing a 50% (w/v) sucrose solution. After preliminary bioassays, the following solutions were tested for oral exposure concentrations: 0.0015, 0.003, 0.005, 0.010, 0.025, and 0.05 ng µL<sup>-1</sup>, and for contact exposure, applied doses were 0.5, 0.8, 0.95, 1, 1.5, and 2.0 ng a.i./bee. Definitive tests followed the same experimental procedure as the preliminary ones. Actara® presented oral LC<sub>50</sub> values of 0.12277, 0.01503, 0.00738, 0.00371, and 0.0021 ng a.i. µL<sup>-1</sup>, and oral LD<sub>50</sub> values of 0.36697, 0.06378, 0.0342, 0.01851, and 0.01159 ng a.i./bee, respectively, for 6, 24, 48, 72, and 96 hours. The contact bioassay LD<sub>50</sub> was 2.16726, 0.96173, 0.76224, 0.62911, and 0.46252 ng a.i./bee, respectively, for 4, 24, 48, 72, and 96 hours. Within the first 24 hours, whether through ingestion of contaminated food at higher concentrations or direct contact of insects with thiamethoxam at higher doses, 80% mortality of bees was observed.

## 2.07.P-Tu168 Method Optimisation for Large Scope Pesticide Multiresidue Analysis in Bee Pollen: a Pilot Monitoring Study

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Despite providing numerous advantages for agriculture, the extensive use of pesticides is known to have negative environmental impact through soil, water, and nontarget plant contamination, but also through animals' and consumers' exposure. In this scenario, the analysis of pesticides in natural products like bee pollen has a cross-sectional value, since it can shed light on pollinators' safety, and it can be a helpful tool for environmental monitoring. Moreover, due to the growing popularity of bee pollen as food supplement, the analysis of pesticides in this bee product is also important for food safety purposes.

The present study focused on the optimization of an analytical method for the evaluation of pesticides contamination in bee pollen. QuEChERS methodology was employed for pesticides extraction, and a new automated clean-up approach for final extracts purification was developed. Subsequently, a mass spectrometry-based method was validated for the targeted analysis of 353 pesticides included in the EU monitoring priority list. The newly developed method was then employed to evaluate the pesticide contamination in 80 bee pollen samples purchased from different countries within and outside the EU. Finally, the results obtained were used to tentatively assess the safety of bee pollen for human consumption following the EU guidelines.

The new automated approach for the clean-up of pesticide extracts from bee pollen proved to be a reliable alternative to the classical clean-up methodologies, and the optimized mass spectrometry-based analytical method was fully validated and applied to assess the presence of a wide range of pesticides with a very low limit of quantification (5 µg/kg) in bee pollen. The sample analysis showed the presence of 77 different pesticides in bee pollen, including several chemicals whose use is no longer allowed. 85% of the samples were contaminated with pesticides, sometimes reaching concentrations higher than the maximum residue levels imposed by the EU in honey and other apiculture products. Nevertheless, the risk assessment showed that consumers are not exposed to an unacceptable risk when consuming the analysed bee pollen.

The experimental methods validated in this study, together with the wide range of data collected on bee pollen contamination with pesticides, represents a step forward towards the comprehension of environmental and food safety threats, and provide a solid basis for future cross-sectional studies.

## 2.07.P-Tu169 New approaches for the analysis of residues of plant protection products in non-typical matrices collected by solitary leafcutter bees (*Megachile rotundata* F.) in a semi-field test design

*Ralph Kirchmaier*<sup>1</sup>, *Arne Mewis*<sup>2</sup>, *Dietke Kleinhenz*<sup>2</sup>, *Julian Fricke*<sup>3</sup>, *Olaf Klein*<sup>3</sup>, *Silvio Knaebe*<sup>4</sup>, *Carmen Gimeno*<sup>5</sup> and *Ignacio Gimeno Rodrigo*<sup>5</sup>, (1)Eurofins Agroscience Services EcoChem GmbH, Germany, (2)Chemistry, Eurofins Agroscience Services EcoChem GmbH, Germany, (3)Eurofins Agroscience Services Ecotox GmbH, Germany, (4)Eurofins Ecotox GmbH, Germany, (5)Eurofins Trialcamp, Spain

In the new EFSA bee guidance document, exposure is a focal point to decide if there is a risk for wild pollinator species through the application of plant protection products (PPPs). So far no standardised protocols are available to determine the transfer rates of a foliar applied PPP to the different bee matrices. In order to provide analytical evidence of the effects of PPPs on wild pollinator species, it is necessary to develop novel methods for preparation, extraction and analysis of those matrices, which are important and closely linked to the biology of these species. Due to the interest in other bee species exposure routes besides nectar and pollen have not been in the focus so far. This information is of utmost importance as there is a difference of exposure of wild bee species to honey bees. Authorities need this information for a proper risk assessment for non-*Apis* bees. In 2022 a first successful attempt was made to supplement a standardised Tier II semi-field test design based on the recommended concept for *O. bicornis* with another bee species (*Megachile rotundata*). In 2023 this test design was refined and a residue sampling component was included for different matrices. The active ingredient chosen for this trial was the fungicide BOS WG 500 (active ingredient boscalid) as it is not toxic to bees and stable in the environment. Residue samples of provision mass (larval diet), nesting material (leaves from brood cells), foliage from clover and nectar and pollen from *Phacelia* flowers were collected. In the laboratory a methodology was developed to analyse foliage from clover and nectar and pollen from *Phacelia*. Furthermore, a standardised methodology was developed to separate nesting material from the provision mass for later analysis. Also an analytical method for provision mass and nesting material (cocoons made of plant leaves) was developed. In order to develop an extraction method which is not only applicable for boscalid, but also to various other PPPs, an approach based on the QuEChERS multi-method was aimed for. Methodological sampling procedures and analytical results for different non-typical matrices will be presented with respect to their potential for the use in risk assessments.

## 2.07.P-Tu170 New experiences and insights of two-year testing with the leafcutter bee *Megachile rotundata* in a semi-field test design

*Julian Fricke*<sup>1</sup>, *Ignacio Gimeno Rodrigo*<sup>2</sup>, *Olaf Klein*<sup>1</sup>, *Carmen Gimeno*<sup>2</sup> and *Silvio Knaebe*<sup>3</sup>, (1)Eurofins Agroscience Services Ecotox GmbH, Germany, (2)Eurofins Trialcamp, Spain, (3)Eurofins Ecotox GmbH, Germany

According to the revised EFSA Bee Guidance Document a point of focus is the risk for wild pollinator species through the application of plant protection products (PPPs). This information is of utmost importance as there is minimal knowledge on exposure of wild bee species. Authorities need this information for a proper risk assessment for non-*Apis* bees. As a

consequence a study design with the solitary red mason bee *Osmia bicornis* L. was developed by the ICPPR non-Apis working group with recommendations for a standardised Tier II test design. With the knowledge on differences in exposure pathways between the solitary bee *Osmia bicornis* and the leafcutter bee *Megachile rotundata*, it is expected that the same PPPs will impact those species differently. So far no standardised protocols are available for solitary bee studies with *Megachile*. The main objective of these two-year tests, conducted in Spain, was to develop a standardized test design based on the recommended design for *Osmia* and to gain further insight into the risk assessment of different routes of exposure by collecting different matrices for residue analysis. *M. rotundata* was released as emerged adults in tunnels containing a bee attractive flowering crop as food and nesting resource (*Medicago sativa* in 2022 and *Trifolium sp.* in combination with *Phacelia tanacetifolia* in 2023). The study included an untreated control and two treatments one sprayed with dimethoate (2022) and the other with fenoxycarb (2023). After application, the bees were exposed not only by the food items but also via contact to the treated crop. This included not only pollen and nectar, but also plant material (leaves) for brood cell construction. Evaluated endpoints were assessed for the whole reproductive period: Establishment of actively nesting females, flight activity observations and the production of brood cells/cocoons. The assessed endpoints were evaluated with respect to their potential for the use in the risk assessment of PPPs. The obtained results are presented and discussed.

#### **2.07.P-Tu171 Definitive Methodology for the Acute Contact Test on the Solitary Bee *Megachiles rotundata*. -LD50 Toxic Reference**

Josep Antoni Aguilar-Alberola<sup>1</sup>, Eugenia Soler<sup>2</sup> and Carmen Gimeno<sup>1</sup>, (1)Eurofins Trialcamp, Spain, (2)Eurofins Trialcamp S.L.U., Alcasser (Valencia), Spain

After carrying out two acute tests with contact application in 2022 (the results were presented in a poster at the ICPPR), the previously developed methodology has been repeated in a new test carried out in 2023. The objective has been to corroborate that the methodology is appropriate for the management and feeding of the study organisms. In addition, the mortality results of the control groups and the LD<sub>50</sub> values obtained using a reference toxicant (dimethoate) have been compared. This allowed us to find out if the values were similar or there may have been a wide variability that did not allow comparable results to be obtained over time. In this test, a mortality of 10 % was recorded in the control group 96 hours after application (in the previous tests, mortality was 6.7 % in both tests). The LD<sub>50</sub> values at 24, 48, 72 and 96 hours for the reference item were very similar in the 3 tests carried out (they varied from 0.179 to 0.237 µg dimethoate/organism). These results show us that the developed methodology is adequate for the maintenance and survival of the study organisms and allows comparable mortality results to be obtained over time within a very narrow margin of variation. Due to this, it is considered that an appropriate methodology is already available to carry out contact acute studies using *Megachiles rotundata*.

#### **2.07.P-Tu172 Selection of an Application Diet for an Acute Oral Test on the Solitary Bee *Megachiles rotundata*.**

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In order to comply with the EFSA requirements regarding studies carried out with solitary bees, a series of preliminary tests were carried out with *Megachiles rotundata* to find out the best diet that can be offered to them. This diet must meet the necessary requirements for the study organisms. These requirements are not only that it contains the necessary nutrients to keep the organisms alive but also that it is palatable enough to feed on that diet and in sufficient quantity to be dosed with the test products. For this test, different types of base diet were used in acclimatization (50 % and 67 % (w/v) aqueous sucrose solutions and pollen paste), to which a small amount of pollen was added or not (except in the case of pollen paste). The groups that had high survival rates were divided into two subgroups, one was without starvation and the other group was starved for 2 hours before exposure. During the exposure phase, the consumption at 6 and at 24 hours were monitored. The results were clear regarding the type of diet. The groups fed with aqueous sucrose solutions with some pollen on the tip of the syringe had high survival rates, while most organisms fed the same diet, but without pollen, were dead within 24 hours. As for consumption, it was very low in all cases during the first 6 hours, regardless of the starvation period. Consumption was acceptable throughout the 24 hours. Due to this, new oral application tests will be carried out using the diets described above, always with the presence of pollen and by monitoring consumption for 24 hours to corroborate the results. Additionally, new ways of providing the food will be tested to find out if it can have any type of effect on the results.

#### **2.08.P Potential Impacts of Anthropogenic Contaminants in the Changing Arctic and Antarctic Ecosystems, Interacting With Other Human Derived Stressors**

##### **2.08.P-Tu173 High Concentrations of Novel Flame Retardants in Indoor Dust from a Cruise Ship in the Arctic**

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The cruise liner industry is the fastest-growing tourism sector. As sea ice decreases and technology becomes more advanced, more cruise ships venture into the once-inaccessible Polar regions. The number of cruise ships –particularly expedition cruises– in the Arctic has increased by 118% between 2012 and 2018. Due to the unique operational environment of cruise liners, stringent fire safety measures are prescribed by the International Maritime Organization (IMO). The International Code for Fire Safety Systems requires that the construction and other material, including textiles, upholstered furniture, and bedding, must pass tests for non-combustibility, fire resistance, flammability, the spread of flame, smoke, and toxicity. In 2023, we collected passive air samples and dust from three expedition cruise ships operating in the North Atlantic and Arctic regions.

The first ship was built in 2002, but renovated in 2020, and is propelled by Marine Biofuel. The second ship was built in 2020 and is a battery-operated hybrid electric ship. The third ship is fueled by Marine Gas Fuel and was built in 2003. All ships have a passenger capacity of approximately 550. The air and dust samples were analyzed for 197 compounds, including 10 PBDE congeners and 24 novel flame retardants. BDE-47 (4.63-25.42 ng/g), BDE-99 (3.88-29.3 ng/g), and BDE-209 (148-2587 ng/g) were detected in 100% of the dust samples. The novel flame retardants, DBDPE (0.13-7.36 µg/g), and DBE-DBCH (159-1317 µg/g) were detected at high levels in 90% of dust samples. TBCO was detected in 62% of dust (6.95- 139.32 µg/g). To our knowledge, the concentrations of DBDPE, DBE-DBCH, and TBCO were higher than most reported indoor dust concentrations. Novel brominated flame retardants have been quantified in Arctic air, soil, and marine biota. More ships are accessing the Polar regions, and may be an additional source of point pollution of harmful chemicals. The presence of high concentrations in a relatively small expedition cruise ship raises concern, considering that other cruise ships can house more than 10 000 passengers (excluding crew). More research is needed to characterize the impact of chemicals used in cruise ship environments on polar regions.

#### **2.08.P-Tu174 Antarctic Threads: Textile Microfibers and Chemical Additives in the wild scallop *Adamussium colbecki* from the Ross Sea**

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Plastic pollution, driven by microplastics and synthetic fibers, poses a threat to Antarctic biodiversity from both global and local sources. Microfibers, released during the use and washing of synthetic textiles, contribute to secondary microplastics and carry associated chemical additives. Despite being considered "The Last Ocean," the Ross Sea faces emerging anthropogenic impacts, including climate change and microplastics. This study addresses the gap in research on chemical additives from synthetic textiles in Antarctic marine organisms, focusing on the scallop *Adamussium colbecki*, a vital species in coastal areas. Further investigation is needed to understand the potential hazards posed by these additives to marine life in the region. This study investigates the presence of textile microfibers in the Antarctic scallop *Adamussium colbecki*, a keystone species of Antarctic coastal areas, along with the content of associated chemical additives as organophosphates (OPEs) and phthalates plasticizers. Specimens from Terra Nova Bay, Antarctica, collected during expeditions in 2004 and 2019, were examined for microplastic contamination and chemical additives. Antarctic scallops showed significant microfiber presence (91.6%, averaging  $4.9 \pm 2.2$  microfibers per individual), mainly black (35%), blue (29%), and red (10%). Chemical analysis identified 15 out of 34 compounds, with prevalent OPEs and notable phthalates. Textile analysis revealed a match between chemical additives in scallops and those in scientific personnel's clothing, suggesting local contamination. Limited analysis prompts further investigation into potential local sources, such as wastewater sewage plants, releasing textile microfibers into Antarctic marine environments.

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#### **2.08.P-Tu175 Exploring Heavy Metal Toxicity in the Antarctic Marine Ecosystem: Investigating the Defensive Role of Metallothioneins in *Trematomus eulepidotus***

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The Antarctic continent, despite its remote location, faces the threat of human-induced environmental changes, including the well-known presence of heavy metal contamination. This study explores the impact of heavy metal pollutants on an endemic Antarctic fish *Trematomus eulepidotus*, specifically examining the role of metallothioneins (MTs) in metal regulation and ROS scavenging. The Antarctic fish, are known to be among the few to express two distinct isoforms of metallothioneins, 1 and 2. In the intricate landscape of metal regulation and defense against environmental stressors, this dual expression emphasizes their extraordinary adaptability to the challenging conditions of their habitat. In this study *T. eulepidotus* specimens were exposed to 100 µg/l of cadmium (Cd) and copper (Cu), and the expression of *mt-1* and *mt-2* genes were analyzed across various organs. Results reveal interesting and distinct organ-specific responses to metal exposure, with the liver and kidney exhibiting elevated MT expression, particularly for *mt-2*. Cadmium exposure induces a significant rise in *mt-1* transcription in the liver, suggesting its potential role in cellular protection against Cd toxicity. In the kidney, both Cd and Cu treatments lead to increased mRNA expression for both MT isoforms, indicating its prominent role in metal detoxification. Brain and gonads responses vary with metal exposure, highlighting the complexity of organ-specific reactions. Metallothioneins, though fundamental, constitute only a portion of the intricate network of the cell detoxification system, necessitating an exploration of

the roles played by both enzymatic and nonenzymatic antioxidant components for a comprehensive understanding. Despite being preliminary, these results advance our understanding of the molecular and functional evolution of Antarctic fishes and lay the groundwork for the potential use of metallothionein expression as well-established biomarkers for metal exposure and oxidative stress. Using Notothenoidea as bioindicator organisms in future biomonitoring campaigns becomes crucial in assessing the anthropogenic impact on the uniquely beautiful yet extremely fragile Antarctic marine ecosystem. Funded by the Italian National Program for Antarctic Research (PNRA), project No. 2018/B2Z1.01

## **2.08.P-Tu176 Assessing the Impact of Penguin Guano on the Antarctic Bivalve *Aequiyoldia eightsii*: Effects on Biomarker Responses**

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Antarctic penguins can recycle Trace Metals (TMs) in the upper layer of the Southern Ocean, via guano production. However, high levels of non-essential metals (e.g. cadmium (Cd), mercury (Hg) and lead (Pb)) have also been reported in guano. During the breeding season, along the Antarctic continent coasts, thousands of pairs of penguins inhabit their colonies, characterized by a huge amount of guano deposited on land. The recycled TMs, either from guano leached into coastal waters or directly released into the sea, could affect benthic communities of shallow Antarctic seawaters, although the effect has not been studied yet. Here, we aimed to study the impact of Gentoo (*Pygoscelis papua*) penguins' guano on the Antarctic bivalve *Aequiyoldia eightsii*, from Livingston Island (South Shetland Islands), one of the most abundant benthic species in Antarctic waters. We analyzed possible oxidative stress and adverse effects in the selected species after exposure to five different guano concentration treatments (from 0 (control) to 6.7 g L<sup>-1</sup>), over a 10-day period. The experiment was ran per duplicate in 25 L plastic aquariums, located outdoor to ensure natural photoperiod and temperature. Four specimens were collected from each treatment on day 2, 4, 7 and 10 for biomarkers analysis. Detoxification metabolism was examined in digestive gland, by the determination of Phase I and II and antioxidant enzymatic activities (Ethoxyresorufin O-deethylase (EROD), Glutathione S-transferase (GST), Glutathione peroxidase (GPX), Glutathione reductase (GR)). Lipid peroxidation (LPO) and DNA damage were measured as biomarkers of effect. The data showed a notable decline in EROD activity over time; a positive induction for GST was observed at day 10. GPX, GR activities, LPO and DNA damage did not show exposure concentration- or time-dependent patterns. Differences in GPX activity and DNA damage were observed between control and guano treatments at day 2 at day 10 respectively. The results suggest that metals released from guano are not significantly adverse for *A. eightsii*, which did not show a clear stress response to guano exposure; however, to the best of our knowledge, no data on these biomarkers in *A. eightsii* are available in the literature. Hence, the data obtained in this study are of great importance for a deeper understanding of the physiology of these Antarctic organisms. Nevertheless, further studies are needed to clarify the impact of penguin guano on marine ecosystem.

## **2.08.P-Tu177 Glacial and urban rivers role in the fate of Contaminants of Emerging Concern in Arctic coastal waters**

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Arctic coastal waters have been subject to high levels of pollution with a wide list of contaminants that can be either delivered with ocean currents and by atmospheric transport from distant regions or may originate from local sources. Terrestrial freshwater input can alter concentration and composition of pollutants in marine water in the coastal zone. Freshwater plumes propagation, driven by local winds and waves, can affect different regions of the fjord, and shape the frontal zones by interacting with saline waters. The aim of this work is to assess the role of the freshwater plumes of glacial and urban rivers in the pattern of pollution in Arctic coastal waters by example of Svalbard fjords. As illustrative Contaminants (iC) in this study, we select polyfluoroalkyl substances (PFAS), microplastics (MPs), and mercury (Hg). PFAS and MPs belong to the group of Contaminants of Emerging Concern (CECs), and Hg to the group of Priority Hazardous Substances. Hg can become a new challenge with regard to climate change due to its release from the thawing permafrost and the change in the sea ice regime. All illustrative contaminants can be transported both by water and by air.

Field studies were performed in populated and unpopulated branches of Isfjorden in July 2023. Water samples were taken in pristine and urban rivers, river plumes and high saline fjord waters for PFAS, MPs, Hg analysis as well as nutrients, organic carbon, and carbonate system. Results showed clear difference of chemical composition in samples for three studied regions representing clean glacial, riverine and contaminated urban waters. Detailed results will be present at the Conference.



## Track 3. Environmental Chemistry and Exposure Assessment: Analysis, Monitoring, Fate and Modeling

### 3.01.A Advances in Bioaccumulation Science and Assessment

#### 3.01.A.T-01 Bioaccumulation Kinetics of Per- and Polyfluoroalkyl Substances in *Pimphales promelas*

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Due to persistence, toxicity and ubiquitous contamination of water resources, there has been growing interest in understanding the disposition of per- and polyfluoroalkyl substances (PFAS) in the environment. However, bioaccumulation dynamics remain poorly understood for many substances within this group of chemicals, and kinetic-based information is lacking across environmental gradients. Therefore, the aim of this study was to examine uptake of 19 PFAS, which were selected at low levels not eliciting standardized adverse outcomes, by fathead minnows (*Pimphales promelas*) over 7 days. The PFAS mixture consists of short and long chain perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonates (PFSAs), a sulfonamide, and fluorotelomer sulfonates. Water, whole-body tissue, and plasma were collected at multiple timepoints, and samples were analyzed via liquid chromatography tandem mass spectrometry. Uptake kinetics were estimated for each PFAS with non-linear regressions for both tissue and plasma. Rapid uptake was observed in both tissue and plasma, reaching pseudo steady-state conditions with 72 – 96 hours. Increasing concentrations of PFAS in tissue and plasma were observed with increasing chain length for both PFCAs and PFSAs. Additionally, ratio based bioaccumulation factors (BAFs), blood:water partitioning coefficients ( $P_{BWS}$ ), and apparent volumes of distribution ( $V_{DS}$ ), were estimated and relationships with chain length were examined. Significant relationships between BAFs,  $P_{BWS}$ ,  $V_{DS}$ , and chain length were observed for both PFCAs and PFSAs. For example, BAFs and  $P_{BWS}$  increased with increasing chain length, while  $V_D$  decreased with increasing chain length. Relationships between PFAS  $V_{DS}$  and BAFs were also examined, and a significant relationship was observed for the PFCAs. These are initial results of an ongoing research effort, and recently, 14-d uptake and 7-d elimination studies of these PFAS in adult fathead minnows at differing pH and salinity conditions were completed. Analytical measurements are ongoing, and additional examination of the relationship between apparent  $V_D$  and kinetic-based BAFs is being performed, which promises to support the development of a predictive understanding for bioaccumulation of PFAS and ionizable organic contaminants, across environmental gradients, in PFAS contaminated sites.

#### 3.01.A.T-02 Significant Bioaccumulation of NSO-Heterocyclic PAHs in *Daphnia Magna* Was Observed Under Controlled Exposure with Passive Dosing

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The ubiquity of heterocyclic polyaromatic hydrocarbons (heterocyclic PAHs) substituted with nitrogen, oxygen, and sulfur heteroatoms (also known as NSO-PAHs), along with their potential for persistence and high toxicity, has raised significant concerns regarding their hazard to aquatic life. Heterocyclic PAHs do not undergo mineralization in the standard ready biodegradability test and even in enhanced tests using adapted microbial communities. Their continuous release could lead to increasing environmental concentrations, thereby exacerbating the risks of adverse effects. However, information on their long-term effects is scarce and little is known about their bioaccumulation in aquatic organisms. Testing such hydrophobic compounds is also not trivial, because (i) the exposure concentration decreases rapidly and dramatically, (ii) they require a long time to reach equilibrium concentration in the exposed organism, and (iii) experimental artifacts often occur due to the use of total water concentrations instead of freely dissolved water concentrations, leading to significant underestimation of bioaccumulation. Given these environmental concerns and to address the testing challenges, the main objective of our work is to develop a reliable experimental design to investigate the bioaccumulation of four heterocyclic PAHs ( $\log K_{OW}$  5.2 to 6.9). We hypothesize that (1) chemical concentrations in water can be kept stable during exposure even at very low levels (ng/L) using a passive dosing method, and (2) *Daphnia magna* can be used for bioaccumulation assessment as it allows quicker uptake and depuration kinetics than e.g. fish and provides sufficient biomass for reliable chemical quantification in the organism. Stable exposure was achieved by using passive dosing at levels as low as 100 ng/L. Only 5 organisms equivalent to 15-35 mg wet weight (depending on the age of the animal) are necessary to quantify body burdens of tested chemicals. All tested heterocycles were very bioaccumulative with logarithm of lipid-corrected kinetic bioaccumulation factor ( $\log BAF_{KL}$ ) above 7. This study provides insights on our understanding of the environmental impact of NSO-PAHs by revealing high bioaccumulation in *D. magna*, and providing valuable insights for enhancing environmental risk assessments and regulatory measures.

#### 3.01.A.T-03 Integrating in Vitro Intrinsic Clearance (OECD TG 319B) for BCF Prediction in a Regulatory Context – Fragrance Chemicals as Case Studies

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Bioaccumulation in aquatic species is a critical endpoint in the regulatory assessment of chemicals which usually involves the determination of the bioconcentration factor (BCF) in fish. *In vitro* intrinsic clearance ( $CL_{IN\ VITRO,INT}$ ) determined in liver S9 fractions (RT-S9) from rainbow trout (OECD Test Guideline (TG) 319B) may be incorporated into appropriate *in silico* models for the prediction of BCFs. Although use of measured  $CL_{IN\ VITRO,INT}$  improves BCF predictions, further confidence

building and understanding of the applicability of the TG 319B and prediction models is needed for regulatory acceptance of this *in vitro* approach.

Here we show the integration of the TG 319B method for 10 fragrance chemicals into an environmental risk assessment as part of REACH dossiers for upgrades or registration of new molecules.

Substrate depletion assays were carried out according to TG 319B with 10 different fragrance chemicals (plus individual isomers) with log  $K_{ow}$  ranging from 4.4 to 8.3. Chemical concentrations were measured by GC-MS. The resulting  $CL_{IN\ VITRO,INT}$  and measured log  $K_{ow}$  were used as input in the revised IVIVE by Nichols et al. and an empirical regression model developed based on *in vivo* BCFs, measured  $CL_{IN\ VITRO,INT}$  in RT-S9 and log  $K_{ow}$  values. For 3 substances, metabolites in RT-S9 were identified by GC-HRMS and LC-HRMS to obtain additional information on the potential metabolic pathways. Due to global registration / RIFM safety assessments, BCFs were subsequently determined *in vivo* for 2 substances.

All fragrance chemicals were significantly biotransformed in RT-S9 with  $CL_{IN\ VITRO,INT}$  ranging from 0.22 to 5.63 mL/h/mg protein. Predicted BCFs were similar with the IVIVE and the regression model and below the EU REACH B criterion of 2000 L/kg. The predicted BCFs with the two different models, a physiological based and an empirical model, were all within a factor of two, thus, increasing the confidence of the predicted BCF values. For the two substances with *in vivo* BCFs, the predicted BCFs were in excellent agreement supporting the good predictability of the *in vitro* approach.

Furthermore, glucuronic acid conjugates were identified as phase II metabolites which may be more easily excreted than the parent molecules confirming the low predicted bioaccumulation potential.

The *in vitro method* TG 319B in combination with two different BCF prediction models is well suited to assess the bioaccumulation potential in fish within a regulatory context.

### **3.01.A.T-04 Application of the Chemical Activity Approach to Assess the Bioaccumulation and Risks of PFAS in Aquatic and Terrestrial Wildlife at PFAS impacted Sites and Ecosystems**

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The presence of Per- and Polyfluoroalkyl Substances (PFAS) at many sites and ecosystems in the EU, US and elsewhere is generally considered to be of high concern because PFAS are known to bioaccumulate in both aquatic and terrestrial organisms and in humans and can exert adverse effects in wildlife and humans. However, there remains considerable uncertainty about the extent to which various PFAS bioaccumulate in wildlife species. As a result, it is difficult to interpret ambient concentrations in terms of risks to wildlife and to develop guidelines for risk management. Some of the difficulties are related to the high degree of ionization of PFAS in the environment; the high affinity of PFAS for proteins and polar lipids; and the fact that exposure and toxicity data are often expressed in different quantities, which prevents an accurate risk characterization, which involves the comparison of exposure and toxicity data. This study explores the application of the chemical activity approach for the development of aquatic and terrestrial bioaccumulation models for PFAS. This presentation discusses the development of a chemical activity meter and its application for bioaccumulation and risk assessment. The chemical activity approach for bioaccumulation assessment is tested by (i) comparing model predicted bioconcentration factors (BCFs, L/kg, wet wt.) of PFAS and measured BCFs obtained from Burkhard et al. 2021; (ii) comparing predicted and observed concentrations of individual PFAS in organisms of (a) the Lake Ontario food web (b) Canadian Arctic marine food web; (c) a piscivorous estuarine food-web and (d) Canadian Arctic terrestrial food web. Finally, the application of the chemical activity approach for risk assessment of PFOS at contaminated sites in the US is discussed. This includes the determination of risk levels in the risk characterization phase and the derivation of concentration based guidelines that can be used for risk management. We conclude that the chemical activity approach is useful for developing bioaccumulation & food-web bioaccumulation models for PFAS and for expressing exposure and toxicity reference data on a common basis, facilitating risk assessment and guideline development.

### **3.01.A.T-05 Temperature Effects on Organic Compound Uptake and Elimination Rates in Aquatic Species**

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Climate change has been driving significant increases in water temperatures. Presence of a wide array of chemicals of emerging concern has been confirmed in various waterbodies around the world. Understanding the dependence of toxicokinetics on temperature in environmental systems is pivotal, spanning from controlled laboratory conditions, typically around 20°C, to the diverse temperatures encountered in the field, ranging across different climate zones from tropical to polar regions. Despite extensive research on individual stressors, the interactions between rising temperatures and chemicals on aquatic species is not well understood.

The aim of this study was, therefore, to bridge this knowledge gap by investigating the relationship between temperature and the uptake and elimination rates of various organic compounds in aquatic animals, predominantly freshwater species.

A comprehensive dataset was compiled to observe the influence of temperature on uptake and elimination rates. We used the Arrhenius equation to establish a correlation between temperature and rates. Furthermore, we adjusted rates to account for organism mass using correlations already established for metabolic rates. Additionally, we integrated octanol water distribution coefficient ( $D_{OW}$ ) to provide chemical-specific insights into uptake and elimination rates.

The application of the linearized version of Arrhenius equation yielded significant relationship between temperature and both, uncorrected and mass-corrected, uptake and elimination rates. Both showed the increase in uptake and elimination rates with increase in temperature. Additionally, mass correction allowed a generalized overview, revealing the role of organism size in toxicokinetics. Analysis of trendlines for individual chemicals across various species showed that chemicals with higher  $D_{OW}$  values exhibit higher uptake rates and, conversely, lower elimination rates compared to those with lower  $D_{OW}$  values.

The convergence of increasing water temperatures and the influx of emerging chemicals in the environment signals potential challenges for aquatic species. This study offers a comprehensive overview of temperature effects on organic compound uptake and elimination rates in aquatic species, elucidating the relationship between temperature, chemical compounds, organism characteristics, and toxicokinetics.

### 3.01.B Advances in Bioaccumulation Science and Assessment

#### 3.01.B.T-01 Advances in Bioaccumulation Assessments of Transformed Nanomaterials Under Environmentally Relevant Conditions

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This study investigates the impact of transformation of engineered nanomaterials (ENM) during wastewater treatment plant (WWTP) processes on their bioavailability and bioaccumulation potential. ENMs released from WWTP by their effluent can accumulate in aquatic sediments.

Bioaccumulation is a concern as this process can increase e.g. ENM concentrations in organisms to a level that can potentially induce effects. *Hyalella azteca*, which is suitable for ENM testing, has been used in several bioaccumulation studies, but previous studies have often overestimated bioavailability by including the gut content during the body burden determination after acid digestion. This is even more problematic for sediment exposure, where the amphipod ingests the sediment.

Isotopically enriched <sup>109</sup>AgENM were synthesized for this study to allow tracking their fate at low environmentally relevant concentrations, overcoming the interference from the natural background.

Sludge from an experimental pilot WWTP (running with municipal wastewater) spiked with <sup>109</sup>Ag and Au ENM was used to spike artificial sediment for the sediment *H. azteca* bioaccumulation tests. With these tests we wanted to test the hypothesis that WWTP processes reduce the bioavailability and bioaccumulation potential of WWTP-transformable (e.g. AgENM) and chemically inert ENM (e.g. AuENM) under realistic conditions.

In our study the bioaccumulation potential will be assessed by measuring the body burden of <sup>109</sup>Ag or Au using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) and single particle ICP-MS (spICP-MS). In addition, the haemolymph of exposed organisms is isolated and analysed by spICP-MS in order to provide insights into the actual bioavailability of the respective ENM. Nano-specific endpoints as recently proposed for the bioaccumulation assessment of ENM using *H. azteca* will be used to exclude ENM-specific artifacts in the BAF determination.

The use of isotopically enriched <sup>109</sup>AgENM is suited for aquatic exposure to follow the fate in different exposure matrices, but also in exposed organisms, despite an elevated natural background. Also, the method of haemolymph isolation and measurement by spICP-MS has been shown to provide valuable data to assess the bioavailability of ENM. The combination of these approaches advances the understanding of the bioaccumulation of WWTP-transformed ENM under environmentally relevant conditions, providing insights into their fate and effects in aquatic ecosystems.

#### 3.01.B.T-02 Biomagnification in Polar Bears: The Role of Interindividual Differences, Dietary Ingestion Rate and the Gut Microbiome

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Some of the most troublesome contaminants biomagnify, i.e., achieve higher fugacities in the tissue of an organism  $f_B$  than its diet  $f_D$ . Thermodynamically, the extent of a chemical's biomagnification is expressed as the fugacity ratio  $f_B/f_D$ , which is called

a biomagnification factor (BMF) and exceeds 1 if the chemical biomagnifies. Over the past few years, we have developed an entirely non-invasive method towards studying the process of biomagnification that relies on the equilibrium sampling in silicone-film coated vessels and chemical analysis of paired dietary and fecal samples. When combined with dietary intake and egestion rates, the approach yields the thermodynamic limit to a chemical's biomagnification in an organism ( $BMF_{lim}$ ). A feces-based biomagnification factor ( $BMF_F$ ) can be estimated, whereby the fugacity in the feces  $f_F$  serves as an estimate of  $f_B$ . To mimic the seasonal change of food availability in the wild, the Toronto Zoo intentionally varies the feeding rate of polar bears throughout the year to induce hyperphagic and hypophagic periods. We determined  $BMF_{lim}$  and  $BMF_F$  for three zoo-housed female polar bears on three occasions with different feeding states, using polychlorinated biphenyls as examples of biomagnifying contaminants. All three bears had very high biomagnification capabilities ( $BMF_{lim}$  was as high as 200) owing to very efficient lipid assimilation, which can reach as high as 99.5%. We found differences in  $BMF_{lim}$  and  $BMF_F$  as high as a factor of 3 between bears. Much of that variability can be explained by the differences in the lipid assimilation efficiency. We further observed indications that a period of starvation can increase the lipid assimilation efficiency. The  $BMF_{lim}$  and  $BMF_F$  of a bear can increase during a period of hypophagia, by as much as a factor of 4. We characterized the gut microbiome in fecal matter using high-throughput 16S RNA gene sequencing to explore its potential influence on lipid assimilation and biomagnification in polar bears. A preliminary result showed the abundance of Enterobacterales is inversely related to the lipid assimilation efficiency and therefore also to  $BMF_{lim}$ ; while the abundance of Bacteroidales does the opposite.

### 3.01.B.T-03 Using An In Silico NAMs Approach To Predict Bioaccumulation In Fish: A Case Study For Anionic Surfactants Within A Regulatory Context

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Bioaccumulation in aquatic organisms is a standard information requirement under EU REACH for registered substances exceeding 100 tonnes/annum. However, studies such as the fish bioaccumulation test (OECD TG 305) require a significant number of vertebrate animals. Ongoing research and regulatory efforts are directed towards establishing reliable New Approach Methodologies (NAMs) such as predictive techniques and frameworks based on *in vitro* tests and *in silico* modelling. Historically, the octanol-water partitioning co-efficient ( $\log K_{ow}$ ) has been used as a screening parameter for determining bioaccumulation potential with defined cut-off values. However, the use of  $\log K_{ow}$  is not considered appropriate for surfactants, due to experimental difficulties arising from their amphiphilic nature. Surfactants often show a stronger affinity for tissues (e.g. proteins, phospholipids) than storage lipids, therefore, the (phospholipid) membrane-water partition/distribution coefficient ( $K/D_{mw}$ ) is proposed as a suitable, more biologically relevant descriptor that has also been able to overcome challenges in experimental determination. New guidance from ECHA suggests the use of  $\log K/D_{mw}$  in conjunction with information on toxicokinetics in aquatic organisms for bioaccumulation assessment of surfactants in a Weight of Evidence (WoE) approach to waive the need for OECD TG 305 vertebrate test. A tiered modelling approach including the use of *in vitro-in vivo* extrapolation (IVIVE) can be used to predict fish BCF of surfactants. One such model, applicable for ionisable chemicals and surfactants is the BIONIC v3 model. By integrating the partitioning behaviour and tissue distribution of a chemical in fish along with information on uptake and elimination kinetics, this model has been shown to produce reliable fish BCF value predictions for several anionic surfactants.

Here, we present a case study for the use of a NAMs approach for assessing bioaccumulation potential within a regulatory context for a series of anionic surfactants (alkyl isethionates). A combination of experimental  $\log D_{mw}$  and experimental *in vitro* biotransformation data (OECD 319B) was used as input to the BIONIC v3 model to demonstrate low bioaccumulation potential which provides WoE to negate the need for *in vivo* fish BCF studies. Challenges and recommendations for future work to strengthen this approach and demonstrate its applicability within a regulatory context will also be discussed.

### 3.01.B.T-04 Bioaccumulation and Trophic Transfer of Tire Particles and Associated Chemicals From Aquatic Invertebrates to Fish

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Tire and road wear particles (TRWP) are produced by the friction between the tires and the road. Data gaps have been identified regarding their fate and potential toxicological impact on the aquatic ecosystem. Benthic organisms could be exposed to TRWP and associated chemicals through direct contact and/or ingestion. While ecotoxicological studies have mainly focused on pelagic organisms, research concerning benthic organisms remains scarce. As 6PPD-Q has been shown to be highly toxic for some salmonids, it is of critical importance to identify the different routes of exposure for fish to tire-associated chemicals. Among benthic organisms, chironomid larvae and gammarids are commonly preyed by fish and could participate in the trophic transfer of TRWP and its associated chemicals in aquatic ecosystems.

Here, we exposed chironomid larvae and gammarids to sediment spiked with 0.5 mass% of cryogenically milled tire tread (CMTT) particles as a proxy for TRWP. After exposure, the invertebrates were collected and subsamples underwent gut depuration before being collected, whereas other subsamples did not. Non-depurated subsamples were used as a surrogate for fish prey in an in vitro digestion scenario. The scenario reproduced the digestion of preys by fish (rainbow trout) for 27 hours using simulated fish digestive fluids. Depurated and non-depurated invertebrates as well as post-digestion gastrointestinal fish fluids were analyzed for organic chemicals and zinc. Pyrolysis-GC/MS analyses were performed on recovered organisms to assess whether ingestion of CMTT occurred. Our results showed that chemicals were transferred to the invertebrates' tissues and biota-sediment accumulation factors could be determined. It was shown that invertebrates ingested CMTT that could accumulate in their guts. Chemical analyses revealed that the tire-associated chemicals were present and bioaccessible in the fish digestive fluids following in vitro digestion of CMTT-exposed invertebrates. Overall, our results (i) showed the bioavailability of tire-associated chemicals for fish feeding on contaminated prey and (ii) highlighted the potential for trophic transfer of both tire-associated chemicals and tire particles along the aquatic food chain.

### **3.01.B.T-05 Surfactant Bioconcentration: The Puzzle Unraveled**

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Assessing the bioconcentration of surfactants is challenging. There are few in vivo data and a strong reliance on predictive tools. However, during the last 3 years in vivo bioconcentration studies for a range of cationic and anionic surfactants in fish have been published, making it possible to develop and evaluate predictive models of surfactant bioconcentration. Here we use the recent developments about surfactant partitioning to develop an interpretive framework to understand how BCF is influenced by surfactant properties (and structure) and environmental pH. We then evaluate this framework using measurements of the BCF and kinetic rate constants of surfactants in fish. Based on a theoretical foundation, equations are developed for the uptake rate constant, the elimination rate constant and BCF as a function of the membrane lipid/water distribution coefficient  $D_{MLW}$ ,  $pK_a$  and pH. The equations are parameterized for a 10 g juvenile rainbow trout and visualized as plots of the output parameters as a function of the neutral fraction of the chemical in the water and  $\log D_{MLW}$ . Good agreement between the modeled output parameters and in vivo measurements was observed. The framework provides a graphical visualization of the influence of physical chemical properties and pH on BCF. It facilitates understanding of the complex interaction of these parameters and provides a mechanistic interpretation. It can be used to predict the bioconcentration behaviour of untested surfactants and to pinpoint key deficits in our mechanistic understanding.

### **3.01.P Advances in Bioaccumulation Science and Assessment**

#### **3.01.P-Th119 The Decotabs as Standardized Food Substrate for Ecotoxicity Bioassays in Gammarus fossarum**

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Feeding rate inhibition is one of the first observed responses when animals are exposed to toxic stress. Its measurement is recognized as relevant tool for studying chemical compounds toxicity under laboratory condition and large-scale aquatic systems quality assessment through active biosurvey. The measurement of feeding rate in *Gammarus fossarum* is currently based on *Alnus glutinosa* leaf discs. However, using leaf discs introduces a risk of chemical contamination of collected leaves, and may be a source of variability in appetency between each leaves sampling. At last, this methodology needs to anticipate the number of experiments and store the leaves which are collected once a year over a very short period of time.

Decomposition and consumption tablets (DECOTABs) represent a surrogate plant litter substitute to study the invertebrate consumption of organic matter in aquatic environments. Recently, DECOTABs have been proposed as substrate for the *Hyalella azteca* Bioconcentration Test (HYBIT). Developing DECOTABs for *Gammarus fossarum* bioassays would make it possible to source and control food composition (nutritional quality and presence of chemical inputs) and standardize its application in biomonitoring programs. Here, we started by investigating during 7 days the kinetic of alimentation on DECOTAB cubes with two different compositions (either TetraMin flake food or shrimp pellets), and we compared the results with those on leaf disks. Food consumption was higher on both TetraMin cubes and leaf discs compared to shrimp cubes, but no difference was observed between leaf discs and TetraMin cubes. Our results suggest that TetraMin DECOTABs are good candidate as substitute of leaf discs. Complementary experiments both in the lab and in the field will help to determine if TetraMin DECOTABs can be employed for ecotoxicity bioassays in *Gammarus fossarum*.

Key-words: ecotoxicology; behaviour; alimentation; survival; gammarids

#### **3.01.P-Th120 HYBIT protocol application in European freshwater amphipods Gammarus fossarum: Case studies with terbutryn and 2,4,5-trichlorophenol**

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The bioconcentration factor (BCF) is the ultimate decisive bioaccumulation criterion as part of the regulatory chemical safety assessment of pollutants. The *Hyalella azteca* Bioconcentration Test (HYBIT) provided a non-vertebrate alternative for fish bioconcentration studies. The aim of this study was to investigate the applying of the HYBIT protocol to other amphipod species, such as the European species *Gammarus fossarum*. To do so, we determined in semi-static conditions the kinetics of bioaccumulation and depuration of terbutryn (herbicide of the triazine family) and 2,4,5-trichlorophenol (bactericidal component) in gammarids by following the HYBIT protocol. Amphipods were exposed to chemicals dissolved in water for 3 days (uptake phase;  $42.2 \mu\text{g}\cdot\text{L}^{-1}$  and  $4.8 \pm 0.2 \mu\text{g}\cdot\text{L}^{-1}$ , respectively for terbutryn and 2,4,5-trichlorophenol), then transferred to controlled conditions for 3 days (depuration phase). Pools of individuals and water samples were collected and analysed at different intervals. First results show that the mortality rate remained below 20% regardless of the treatment (14.2% and 9.2%, respectively for terbutryn and 2,4,5-trichlorophenol), which meets one of the validity criteria of the HYBIT protocol. Water and gammarid tissue analyses allowed us to calculate BCFs values (BCF = 0.8 and 162 respectively for terbutryn and 2,4,5-trichlorophenol) which were compared to those in *H. azteca*. While BCFs were relatively similar in both species regarding 2,4,5-trichlorophenol, BCF was significantly lower in *G. fossarum* than in *H. azteca*. Overall, our study suggests that HYBIT protocol can be applied to a European amphipod species but also highlights that molecules can bioaccumulate differently in relatively similar species, making model predictions more difficult than expected.

Key-words: ecotoxicology; laboratory exposure; bioconcentration factor; gammarids

### 3.01.P-Th121 Uptake of Venlafaxine and its Metabolite O-Desmethyl-Venlafaxine in Lettuce under Hydroponic Conditions

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The presence of antidepressants like Venlafaxine (VFX) and its metabolite O-desmethyl-venlafaxine (OD-VFX) in urban waters and reclaimed water presents concerns regarding their impact on organisms and the food chain, particularly in agricultural water reuse. This study investigates VFX and OD-VFX uptake and translocation in lettuce under hydroponic conditions, considering enantioselectivity. In a controlled hydroponic system, four treatment groups were studied: R/S-Lettuce-VFX, R/S-Lettuce-OD-VFX, Control-Lettuce, and Abiotic-Control. Solutions for each treatment, of 100  $\mu\text{g}/\text{L}$  (50  $\mu\text{g}/\text{L}$  each enantiomer) were prepared, renewed twice weekly, and sampled at 0, 2, and 4 weeks. Water samples were directly injected, while lettuce samples underwent bead-beating/SPE extraction and UPLC-QqLIT analysis. Both compounds were absent in the control hydroponic solution. Reduction in concentrations occurred in abiotic conditions, while higher concentrations in the output of lettuce systems were observed. Enrichment of R-enantiomer in OD-VFX treatments suggests aerobic degradation of the S-OD-VFX. Growth of lettuce was unaffected by the presence of the drugs. VFX and OD-VFX uptake occurred in roots and shoots at 2 and 4 weeks. OD-VFX exhibited higher uptake in roots, possibly due to its increased hydrophilicity, indicating facilitated transport. Root concentrations were consistently higher than shoots. OD-VFX showed lower translocation factor values, suggesting greater retention in roots than VFX. Enrichment of S-VFX in shoots aligns with reported preferential metabolism of R-VFX. The study revealed a chiral selectivity, with a higher transformation of R-enantiomers to metabolites. Notably, both drugs reached aerial lettuce tissue, emphasizing the need for future studies considering environmental concentrations and polluted water exposure in hydroponic conditions. In summary, VFX and OD-VFX uptake in hydroponically grown lettuce, primarily in roots, displayed chiral selectivity with metabolite transformation. Understanding their behavior under varying environmental concentrations is crucial for assessing their risks in agricultural settings utilizing polluted waters.

### 3.01.P-Th122 Bioturbation Affects Bioaccumulation of Per- and Polyfluorinated Substances (PFAS): Uptake from Sediments by a Rooting Macrophyte and a Benthic Invertebrate

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Per- and Poly Fluorinated Substances (PFAS) are a widely used group of anthropogenic compounds, with a multitude of industrial and consumer applications. The widespread use of PFAS together with their persistence and bioaccumulation potential has led to global contamination of the abiotic and biotic environment. Nevertheless, their distribution across environmental compartments, including the potential uptake by aquatic plants, remains largely understudied. The aim of the present study was therefore to investigate the presence of PFAS from different chemical classes in sediments, to assess their bioaccumulation in an aquatic plant and a benthic invertebrate, and to examine the effects of bioturbation by a sediment dwelling invertebrate on the distribution and uptake of PFAS by the plants. To this end, sediments originating from a heavily PFAS-contaminated and a reference site were collected. Rooting macrophytes (*Myriophyllum spicatum*) were exposed to these sediments under laboratory conditions. After 28-days, in half of the replicates, a sediment dweller (*Lumbriculus variegatus*) was introduced and left there for another 28 days. At the end of the 56-day experiment, PFAS concentrations were quantified

in sediment, water, roots, shoots, and worms. The bioaccumulation of a variety of PFAS in the plants was determined in the presence and absence of the worms. In total 21 and 29 out of the 43 PFAS screened for were detected in the reference and contaminated sediments, respectively, with the concentrations and profiles varying significantly between the sediments. Several of these compounds were also found in the worms and almost all were detected in the *M. spicatum* tissues, with the sum of PFAS concentrations by far exceeding that in the original sediments. The PFAS distribution over the different compartments was affected by the presence of the worms, significantly reducing the PFAS concentrations in the plant tissues. This effect was more evident for the precursors, suggesting potential (bio)transformation of these compounds into their sulfonic acid end products when worms were present. Our results highlight that a wide spectrum of PFAS detected in aquatic environments can be taken up by macrophytes and benthic invertebrates and that the presence of a sediment dweller can affect PFAS distribution and bioaccumulation in aquatic ecosystems.

### **3.01.P-Th123 Trophic Transfer of Halogenated Organic Pollutants in a Wetland Food Web: Insights from Compound-Specific Nitrogen Isotope of Amino Acids and Food Source Analysis**

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Halogenated organic pollutants (HOPs) in wetlands are of particular concern because of their adverse effects on wetland biota. However, owing to the limitations of food source analysis techniques and trophic position (TP) quantification techniques (bulk-N-SIA), studies on HOPs trophic transfer have mainly focused on single aquatic food chains or terrestrial food chains, whereas few studies have been conducted on HOPs bioaccumulation and biomagnification behavior in wetland food webs, in which aquatic and terrestrial food chains interact. To address the above issues, this study developed a trophic position model (TP<sub>mix</sub> model) using compound-specific nitrogen isotopic analysis of amino acids (AAs-N-CSIA) with multiple food source analyses (simmr and QFASA models). This model simultaneously considered a food web with diverse consumers and primary producers and was applied to construct a wetland food web in Southern China. We also investigated the trophic transfer of HOPs in food webs.

By comparing the plausibility of the feeding relationships between organisms and the reliability of the quantified TMFs of HOPs in the wetland food web, we found that the TP<sub>mix</sub> model more accurately characterized the structure of the wetland food web and significantly improved the reliability of the TMFs compared to the TP<sub>bulk</sub>, TP<sub>AAs</sub>, and TP<sub>simmr</sub> models. Food source analysis revealed that the food web consists of three interlocking food webs (kingfisher, crab, and amphibian (frogs)). The TMF calculated using TP<sub>mix</sub> in the three food webs was significantly higher than that calculated using TP<sub>bulk</sub>, indicating that TP<sub>bulk</sub> seriously underestimated the HOPs biomagnification capacity. Additionally, the parabolic trends of TMF<sub>mix</sub> across combinations of log *K*<sub>OW</sub> in the aquatic food web (kingfisher and crab) were similar to those reported in previous studies. However, the parabolic trends of lg TMFs<sub>mix</sub> across combinations of log *K*<sub>OW</sub> in amphibian (frog) food web were distinct from those of aquatic food webs (kingfisher and crab), which may be related to the differences in the biological composition of the food web and HOP bioaccumulation pattern between aquatic and terrestrial organisms. In summary, this study provides a new tool to accurately study the trophic transmission of HOPs in wetlands and terrestrial food webs with diverse species and complex feeding relationships.

### **3.01.P-Th124 Bioaccumulation, translocation and biomagnification of tetrabromobisphenol A and hexabromocyclododecane in mangrove wetlands from South China**

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Tetrabromobisphenol A (TBBPA) and hexabromocyclododecanes (HBCD) are of ecological concern due to their ubiquitous presence and adverse effects. There is a paucity of data on environmental fate of such compounds in mangrove wetlands. In this study, mangrove sediments, plants and biota were collected from the Pearl River Estuary (PRE) and Jiulong River Estuary (JRE) in South China to investigate bioaccumulation, translocation and biomagnification of TBBPA and HBCD.  $\gamma$ -HBCD and  $\alpha$ -HBCD was the two main diastereoisomer of HBCD in mangrove sediments. Sediments were selectively enriched for (-)- $\gamma$ -HBCD. TBBPA concentrations in mangrove sediments from Guangzhou rose during 2012-2015 and declined from 2015 to 2021. HBCD concentrations in the PRE mangrove sediments exhibited an increasing trend from 2012 to 2021. Tissue-specific accumulations were observed, varying with plant species and compounds. HBCD diastereoisomer patterns were similar for all plant species.  $\gamma$ -HBCD was the major diastereoisomer in roots, while  $\alpha$ -HBCD dominated in stems and leaves. The predominance of  $\alpha$ -HBCD in aboveground tissues may be ascribed to diastereoisomer-specific translocation, isomerization and/or metabolism in mangrove plants. Preferential enrichment of (-)- $\alpha$ -, (-)- $\beta$ - and (+)- $\gamma$ -HBCD was found in all mangrove plant tissues, suggesting the enantioselectivity for HBCDs in mangrove plants. Translocation factors of HBCD diastereoisomers and log *K*<sub>ow</sub> were negatively correlated, indicating passive translocation of HBCD, driven by water movement during transpiration. Sediment-root bioaccumulation factors and log TFs both showed no obvious correlation with log *K*<sub>ow</sub> of HBCD diastereoisomers.  $\alpha$ -HBCD was dominant in the mangrove biota. A significant enrichment of (-)- $\alpha$ -HBCD was found in the biota. Bioaccumulations were seen for TBBPA and  $\alpha$ -HBCD as their biota-sediment accumulation factors were greater than 1. (-)- $\alpha$ -HBCD had significantly greater BSAFs than (+)- $\alpha$ -HBCD, indicating the preferential bioaccumulation of (-)- $\alpha$ -HBCD. Biomagnification factors of TBBPA ranged from 0.83 to 1.51, which varied among feeding relationships and mangroves. Positive relationships were found between TBBPA concentrations and trophic levels of the biota species with trophic magnification factors of 2.17 for the PRE and 1.22 for the JRE, suggesting that TBBPA biomagnifies in the mangrove food webs. No biomagnifications were observed for SHBCD,  $\alpha$ -HBCD and its enantiomers.

### **3.01.P-Th125 Optimization of a deep learning-based automated sorting robot for bioconcentration studies with the fresh amphipods *Hylella azteca***

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Abstract

Bioconcentration factors are usually determined by fish flow-through tests performed according to the OECD 305 technical guidance (OECD, 2012) that are time consuming, expensive, and consumer of a large number of laboratory animals. Alternative test design using the freshwater amphipod *Hylella azteca* for bioconcentration studies has been recently developed and highlighted a high potential with good correlation with fish BCF values. For bioconcentration studies using *H. azteca*, male amphipods should be preferred to female organisms. The manual sexing of male adult amphipods is obviously time consuming and requires care and skills. A deep learning-based automated sorting robot has been developed for *H. azteca* by the Life Science Methods company (<https://www.lifesciencemethods.com>). Nevertheless, for experimental reasons, an anesthesia step is necessary prior to the automatic selection. In this study, we showed that a single-pulse of 90 minutes of tricaine at the concentration of 1 g/L can be used and recommended for further studies since it allows time to select *H. azteca* males manually or automatically using the robot without any harmful effect to the organisms. In a second part we exhibited that the robot has the ability to select, sort and disperse the males of a whole batch of *H. azteca* as efficiently as manually but quicker with less risk to damage or stress the living organisms. In a last part of the study, the bioconcentration factor of two well-known organic substances (terbutryn and prochloraz) combining or not the use of an anesthetizing step, the automated selections of male and the HYBIT (*H. azteca* bioconcentration test) protocol was evaluated. The different BCFs values obtained in this study for prochloraz and terbutryn were in accordance with those indicated in the literature and showed that an anesthetizing step had no effect on the BCFs values. Therefore, these data validated the utility and interest for this novel gender-based sorting machine developed by life sciences methods for selecting males in order to perform bioconcentration studies with *H. azteca*. Based on these initial results, and considering technological developments, the automated sorting robot is currently being further developed to improve sorting speed and enable sorting on the basis of developmental and phenotypic criteria for ecotoxicity tests associated with these bioconcentration tests under the HYBIT guideline

### **3.01.P-Th126 Assessing Bioaccumulation with the Biomagnification Factor (BMF)**

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The Bioconcentration Factor (BCF) has played a key role in identifying the bioaccumulation potential of organic substances in aquatic organisms. However, the BCF has some key limitations, including its onerous and difficult measurement in laboratory tests (especially for very hydrophobic substances); limited domain of applicability (i.e. water breathing organisms only); and lack of thermodynamically or biologically relevant criteria values that can be used to determine if a substance is truly bioaccumulative. It has been proposed that the biomagnification factor (BMF) is a more generally applicable metric (i.e. applies to water and air-breathing organisms) with thermodynamically and biologically relevant criteria values, that can be measured in laboratory based tests that are simpler than BCF tests and can also be derived from field data. One of the key questions for using the BMF in bioaccumulation assessments is how the BMF compares to the BCF and what this means for bioaccumulation assessment. To address this question, we compiled data from 258 dietary and 350 aqueous bioaccumulation studies in fish to provide a data base for the BCF and the BMF for the same chemical and the same fish species. This data base was used to investigate (i) the application of a linear regression relationship between the BCF and the BMF; and (ii) the application of the ADME-B (bioaccumulation) approach for regulatory bioaccumulation assessment. The data indicate a weak correlation between the BCF and the BMF for all data combined. This suggests that any correlation between the BCF and BMF is dependent on the chemicals selected. Comparing BCFs derived from dietary bioaccumulation tests using the ADME-B approach are in reasonable agreement with independently observed BCFs in the same fish species. The ability to derive both BCF and BMF data from the results of dietary bioaccumulation tests provides a weight-of-evidence approach where based on the BCF and BMF values for the substance, the substance can be categorized as non-bioaccumulative, bioaccumulative and very bioaccumulative.

### **3.01.P-Th127 Bioaccumulation of Caffeine in Organisms of Different Trophic Levels**

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Caffeine is a natural constituent of more than 60 species of plants. It is found in the daily diet in beverages such as coffee, tea, chocolate, energy drinks and some soft drinks. It is considered the most consumed stimulant substance worldwide. It has been detected in concentrations between 1 and 3,293 µg/L in natural waters and treatment plants, since it is highly soluble in water. There are few studies on the effects that this compound can cause in aquatic organisms. For all of the above, the objective of this work was to evaluate the accumulation of caffeine in tissues of aquatic organisms of different trophic levels, the microalgae *Monoraphidium pusillum*, the cladoceran *Daphnia magna* and the fish *Danio rerio*. Bioassays were carried out where the organisms were exposed to four concentrations of caffeine (0.025, 0.05, 0.1 and 0.5 mg/L); the microalgae and cladocerans were exposed for 5 days and the fish for 10 days. At the end of the exposure period, the extraction was carried out



with a water-ethanol mixture (1:1), by means of sonication. The extracts were analyzed by fluorometry (262 nm) to determine the caffeine concentration. In the tests carried out with the microalgae, it was observed that the bioaccumulation factors varied from 1.6 to 15.8. In the bioassays with cladocerans, values of 3.4 to 7.5 were obtained and in the tests with fishes the bioaccumulation factors varied from 1.4 to 4.1.

### 3.01.P-Th128 Biodistribution of europium-doped polystyrene nanoplastics in terrestrial crustacean *Porcellio scaber*

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The biodistribution of nanoplastics in organisms is currently largely unknown. One of the options is to track nanoplastics labeled with metals, such as for example europium (Eu). We investigated the biodistribution of two Eu-doped polystyrene nanoplastics (EuPSs) with different sizes ~ 100 nm, and 300 nm in woodlice *Porcellio scaber*. Dissolution of Eu from EuPSs in deionised water was negligible (0.9% and 0.0002% for 100 nm and 300 nm, respectively). Water suspension of EuPSs (10 µg Eu/g dry leaf) was applied onto the hazelnut leaves upper surface, and fed to woodlice for 14 days. In addition, a positive control, EuCl<sub>3</sub> was tested (10 and 100 µg Eu/g dry leaf). We used three approaches to track Eu in woodlice: the total Eu in the gut, hepatopancreas (digestive gland), and rest of the body using inductively coupled plasma mass spectrometry (ICP-MS), laser ablation (LA) ICP-MS to investigate the distribution on whole animal sections (chemically fixed using modified Karnovsky fixative and embedded in paraffin) and X-ray microtomography (micro-CT) on whole animals (fixed and dried using standard protocols for scanning electron microscopy). Both ICP-MS measurements and LA-ICP-MS revealed that most of Eu remains in the gut when animals are exposed to EuPSs. However, some Eu was also detected in the rest of the body (potentially the body surface) and in the digestive gland. The total concentration of Eu in the gland as measured by ICP-MS in the case of EuPS exposures was comparable to those of the lowest concentration of EuCl<sub>3</sub> salt (10 µg Eu/g dry leaf), but a much higher concentration of Eu was measured in the case of the highest concentration of EuCl<sub>3</sub> salt (100 µg Eu/g dry leaf). This is the first study of using the micro-CT to map Eu in whole organisms. We managed to obtain representative 3D images of the internal anatomy of the animals and signals indicating the presence of Eu mainly in the gut. Although the unanimous interpretation of the distribution of the doped nanoplastic in the animal was obscured by a relatively low Eu signal, the detection of EuCl<sub>3</sub> (100 µg Eu/g dry leaf) in the hindgut epithelium proved the potential of micro-CT analysis in similar studies. Overall these results indicate that Eu was assimilated in the glands of woodlice. Either Eu dissolved from EuPSs in the acidic digestive tract or EuPSs entered the lumen of the glands. This will be investigated further with more specific methods.

### 3.01.P-Th129 Species-specific prey uptake and biotransformation of chiral polychlorinated biphenyls in riparian and aquatic food webs

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The atropisomeric enrichment of chiral polychlorinated biphenyls (PCBs) can trace the movement of PCBs by food webs, but it is a challenge to elucidate the prey uptake and stereoselective biotransformation of PCBs in different species. The concentrations and enantiomer fractions (EFs) of chiral PCBs in invertebrates, fishes, amphibians, and birds were investigated. Chiral PCB signature was estimated in total prey for different predators based on quantitative prey sources. The nonracemic PCBs in snakehead (*Ophiocephalus argus*) were mainly from prey. EFs of PCBs in amphibians and birds were mainly influenced by biotransformation, which showed enrichment of (+)-CBs 132 and 135/144 and different enantiomers of CBs 95 and 139/149. Biomagnification factors (BMFs) of chiral PCBs were higher than 1 for amphibians and passerine birds and lower than 1 for kingfisher (*Alcedo atthis*) and snakehead. BMFs were significantly correlated with EFs of chiral PCBs in predators and indicative of atropisomeric enrichment of PCBs among different species. Trophic magnification factors (TMFs) were higher in the riparian food web than in the aquatic food web due to the high metabolism capacity of chiral PCBs in aquatic predators. The results highlight the influences of species-specific prey sources and biotransformation on the trophic dynamics of chiral PCBs.

### 3.01.P-Th130 Development and Evaluation of Aquatic and Terrestrial Food Web Bioaccumulation Models for Per- and Polyfluoroalkyl Substances

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There is a need for reliable models to predict the food-web bioaccumulation and assess ecological and human health risks of per- and poly-fluoroalkyl substances (PFAS). In the present study, we (i) develop food-web bioaccumulation models for PFAS and (ii) evaluate the performance of the model using available laboratory and field data. We employ a simple mass balance modelling approach, which has proven useful for modelling non-polar lipophilic contaminants in aquatic and terrestrial food webs. For the current PFAS models, we use six different distribution coefficients to represent equilibrium partitioning of a given PFAS in organisms and derive rate constant ( $k$ ) values. These include distribution coefficients for albumin-water ( $D_{ALB-W}$ ), transporter protein-water ( $D_{TP-W}$ ), structural protein-water ( $D_{SP-W}$ ), neutral lipid-water ( $D_{NL-W}$ ), phospholipid-water ( $D_{MW}$ ), and carbohydrate-water ( $D_{CW}$ ). The models are designed to predict internal whole-body and tissue-specific concentrations ( $C_B$ , ng/g) and activities ( $a_B$ , unitless) of individual PFAS and can be applied to aquatic (freshwater, marine) and terrestrial food webs. Chemical activity, ( $a$ , unitless) is related and can be approximated by the chemical concentration ( $C$ , mol/m<sup>3</sup>) and solubility or sorptive capacity ( $S$ , mol/m<sup>3</sup>), i.e.,  $a = C/S$ . A chemical-activity-based biomagnification factor (BMF) is

determined as the ratio of the PFAS activity in the predator ( $a_B$ ) and its diet ( $a_D$ ), i.e.,  $a_B/a_D$ . A predator-diet chemical activity ratio  $> 1$  indicates the occurrence of biomagnification. Results show that model predictions of laboratory measured bioconcentration factors (BCFs, L/kg) and field based bioaccumulation factors (BAFs, L/kg) of PFAS in fish were in good agreement with observed data. For example, mean model biases (MB, ideal MB = 1) for predicted whole-body BCFs of PFAS was 0.98. Model predicted concentrations of PFAS in aquatic and terrestrial food webs were also in reasonable agreement with empirical observations. Modelling results indicate food web specific magnification of PFAS, with the highest degree of biomagnification occurring in food webs comprised of air-breathing wildlife. Albumin-water, structural-protein-water, membrane-water distribution coefficients ( $D_{ALB-W}$ ,  $D_{SP-W}$ ,  $D_{MW}$ ) and renal clearance rate ( $CL_R$ ) are among the most important model parameters. With further development and testing, these mechanistic models may be useful for future PFAS screening and risk assessment initiatives.

### **3.01.P-Th131 Metabolic activities in Rainbow trout (*Oncorhynchus mykiss*) S9 fractions from liver and extrahepatic organs as an alternative in vitro ecotoxicity assessment approach**

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Whole body biotransformation rate constants can be calculated using an appropriate *in vitro* to *in vivo* extrapolation (IVIVE) model. These models use  $CL_{IN VITRO, INT}$  rates derived with OECD Test Guideline 319B or 319A to estimate liver clearance rates, which are then extrapolated to a whole-body (*in vivo*) biotransformation rate constant. However, beside the liver, extrahepatic organs may also display Phase I and Phase II biotransformation activities and thereby play a role in metabolic clearance and bioaccumulation of compounds. To address these questions, we have maintained rainbow trout (*Oncorhynchus mykiss*) under controlled housing conditions according to OECD 319A/B. Specimens of eight sexually immature animals were harvested and pooled, including liver, gill, intestine, brain, heart and spleen. S9 fractions were prepared to determine the Phase I and Phase II enzyme activities by Liquid Chromatography-Mass Spectrometry analysis. Cytochrome P450 activities, glucuronidation and sulfatation activities were analyzed. The liver displayed the highest Cytochrome P450 activities of all organs tested. Choxoxazone Hydroxylase activity was only detectable in liver, 1-OH-Midazolam Hydroxylase activity was mainly restricted to liver, minor activities could be detected in intestine. However, Phenacetin-O-Deethylation was also detectable in the intestine (34%), gill (16%) and spleen (11%) compared to total enzyme activity. Diclofenac-hydroxylase activity was present in all organs, as well as Bupropion-4-Hydroxylase activity, which was more or less evenly distributed among all organs. Phase II activities were detected in the liver, gill, intestine and heart, but not in spleen or brain. In summary, the liver is the major organ for detoxification of compounds. However, extrahepatic organs, mainly intestine and gill, but also the brain, heart and spleen exhibit substantial cytochrome P450 activities. Phase II enzyme activities were also detected in the intestine and gill. Our results suggest that extrahepatic organs, mainly intestine and gill, should also be taken into account when bioaccumulation and *in vitro* clearance rates are determined for IVIVE modeling in rainbow trout.

### **3.01.P-Th132 A Food Web Bioaccumulation Model for Quantifying the Dietary Exposure to Persistent Organic Pollutants of Beluga Whales from the St. Lawrence Estuary, Quebec, Canada**

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The elevated levels of persistent organic pollutants (POPs) in beluga whales (*Delphinapterus leucas*) of the St. Lawrence Estuary (SLE), classified as an endangered species, may be linked to their current population stagnation, despite many recovery efforts. However, a quantitative assessment of the bioaccumulation of POPs in the aquatic food web leading up to the SLE beluga whales has not yet been done. Therefore, as part of the ongoing effort in their population conservation, further understanding the diet of the SLE belugas and modeling the bioaccumulation of contaminants through their food web may help shed light on the dietary exposure pathways of these whales. Here, we construct a food web with feeding relationships and diet compositions of male and female SLE beluga whales using previously reported stomach contents data and staple isotopic ratio measurements (i.e.,  $\delta^{13}C$ ,  $\delta^{15}N$ ) in beluga muscle tissue. Eleven potential prey species of the belugas were identified, whose feeding relationships, diet composition, and trophic position were estimated from the diets reported for each species in the literature. Using these feeding preference matrices of the aquatic food web and organism-specific characteristics collected from the literature, we parameterized a previously published food web model, which utilizes a mass balance approach to calculate steady-state concentrations of lipophilic chemicals in each of the organisms. The final assembled food web model includes phytoplankton, zooplankton (copepods and krill), seven invertebrate species, and nine fish species. We applied the model to quantify the bioaccumulation of several classes of POPs, including polychlorinated biphenyls, perfluoroalkyl sulfonamides, and halogenated flame retardants in the prey species of the SLE belugas. We also compared the predicted results with measured concentrations of these contaminants in the aquatic species of the SLE to evaluate the model performance, which consisted of analyzing 189 samples of the SLE prey species for all compounds of interest.

### **3.01.P-Th133 Enabling Regulatory Confidence in the Reliability of Stable Isotope Data Used to Describe the Trophic Position of Organisms in Bioaccumulation Studies**

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An important environmental priority under the United Kingdom's Registration, Evaluation, Authorisation and restriction of Chemicals (UK REACH) regulations is the identification of persistent, bioaccumulative and toxic (PBT) and very persistent,

very bioaccumulative (vPvB) substances. Assessment of a substance's potential to bioaccumulate is usually determined from a measured bioconcentration factor in an aquatic species, in laboratory studies (e.g. according to OECD Test Guideline 305, 2012).

However, other scientific information can be used as part of a weight-of-evidence approach. This can include information on the ability of a substance to biomagnify in a food chain. An increasing number of publications use stable isotopes of carbon ( $^{12}\text{C}$  and  $^{13}\text{C}$ ) and nitrogen ( $^{14}\text{N}$  and  $^{15}\text{N}$ ) to describe the trophic position of organisms. When combined with data on the concentration of a substance in those organisms, biomagnification factors (BMFs) and/or trophic magnification factors (TMFs) can be calculated.

Very little regulatory guidance is available on the measurement and interpretation of stable isotope data, which reduces confidence in derived BMF/TMFs. This study aimed to address this by systematically reviewing: 1) best practice and recommendations for isotopic analysis across multiple disciplines; and 2) natural and anthropogenic sources of variation within stable isotope measurements.

Search strings were run on both "Scopus" and "WebOfScience" in July 2023. The results were combined (n = 2049) and duplicates (n = 805) removed, with 1244 discrete sources subjected to primary and secondary screening. Best practices were identified across sample collection, preparation, calibration, analysis and correction, and reporting stages. Potential sources of variation identified included: organism sex, size, age, specific tissue sampled, population sampled, season, climate and anthropogenic influences.

A draft guidance document was produced to facilitate assessments of stable isotope data, increasing regulatory confidence in the robustness and reliability of those data. In turn, this will facilitate the use of BMF/TMF studies in decision making. In addition to regulators, this work will be of interest to researchers at the inception stage of a bioaccumulation study that will rely on stable isotope data.

### **3.01.P-Th134 Temperature Mediated Bioconcentration and Behavioral Effects of Mirtazapine in Aquatic Invertebrates**

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Pharmaceuticals represent an important class of emerging contaminants that has been detected in surface waters worldwide, and due to their continuous input in the recipients via sewage treatment plant effluents, they have been recognized as pseudo-persistent pollutants. Because of their biological activity at very low concentrations and the fact that most of the drug target receptors have been evolutionary conserved across taxa, the risk of inducing detrimental effects in non-target aquatic organisms is not negligible. Those can include impairment in development, morphology, physiology, metabolism, and reproductive traits. On top of that, the pharmacological effects similar to those in human patients might be expected, for example changes in organism's natural behavior as a result of exposure to neuroactive pharmaceuticals. It is generally assumed that non-target aquatic organisms deal with the pharmaceuticals similarly to humans, i.e. the parent drugs are being metabolized via phase I and II biotransformation pathways and subsequently excreted. However, differently from humans, the biological processes of ectothermic aquatic organisms are significantly affected by the temperature of their environment, which most likely also affect the detoxification mechanisms. In the present work, we have focused on how the temperature affects bioconcentration and biotransformation of commonly detected antidepressant mirtazapine in aquatic larvae of two macroinvertebrate species - *Aeshna juncea* and *Coenagrion hastulatum*. We have exposed these experimental organisms to  $1 \mu\text{g L}^{-1}$  of mirtazapine at  $10^\circ\text{C}$  and  $20^\circ\text{C}$  for seven days and then analyzed the parent drug and its main metabolite desmethylmirtazapine in each individual. In addition, both studied species have been subjected to behavior trial prior sampling to assess potential effects of mirtazapine on foraging and activity, representing important fitness related behavior traits. The results of our study show significant difference in ability to metabolize the studied drug between the selected species and a strong influence of temperature on bioconcentration in both organisms. Further, we have confirmed the effect of mirtazapine on studied behavior, which was disproportionally regulated by the temperature treatment as well. Our study underlines the need to study the adverse effects of emerging contaminants in the perspective of other environmental stressors.

### **3.01.P-Th135 Optimization of Avian In Vitro Substrate Depletion Assays to Study Biotransformation of Organic Chemicals**

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Avian species play a critical role in aquatic and terrestrial ecosystems but are understudied with regard to bioaccumulation assessment, which historically has focused on aquatic species. Bioaccumulation in fish is often assessed using bioconcentration factor (BCF), a metric which is assumed to be protective of both aquatic and terrestrial organisms. In air breathing organisms such as birds, biomagnification factor (BMF) describes transfer of accumulated chemicals across trophic levels. Historically,

these metrics have been determined using costly *in vivo* testing in fish and rat models, with relatively limited work using birds outside of field studies. Recently, it has been suggested that a kinetic approach that views these metrics as the product of competing rates of whole-body uptake and elimination may be refined by incorporating tissue specific biotransformation rates derived from substrate depletion assays using isolated hepatocytes or liver S9 subcellular fractions. The present study seeks to bring avian biotransformation assays to a similar level as fish by optimizing and expanding the use of *in vitro* substrate depletion assays. Initial optimization efforts have focused on methods of isolation and cryopreservation, as well as assay conditions using mallard duck (*Anas platyrhynchos*) as a model species. *In vitro* intrinsic clearance rates of the benchmark biotransformation chemical pyrene, as well as 4-*n*-nonylphenol were determined across a variety of assay conditions including pH, temperature, and buffer composition. Cryopreservation and thawing protocol optimizations were investigated using commercially available, as well as freshly isolated hepatocytes and subcellular S9 fractions. Further research will apply these optimized assays to ten organic chemicals, including PAHs, PCBs, bisphenols, organophosphates, and neonicotinoids to generate high-quality substrate depletion data. This work also provides an opportunity to identify metabolites and evaluate biotransformation pathways and enzyme activities. The optimized assay will be applied to two additional species of birds, selected based upon ecological relevance, practicality, and life history traits to contribute to a robust database of biotransformation data. The prospective goal of this study is to create the foundation for a future ring trial or similar application to further inform avian wildlife risk assessment with regard to bioaccumulation.

### 3.01.P-Th136 Assessment of Methods for Determining The Membrane-Water Partition Ratio for Surfactants

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Surfactants are often classified as difficult to test chemicals (OECD Guidance 23), which may lead to uncertainty in their environmental fate and behaviour. A key factor hampering adequate fate modelling is that octanol-water partition coefficients (Kow) are often highly uncertain for surfactants, and poorly representative of the interaction energy of ionic surfactants with soil and tissue matrices. The phospholipid membrane-water partition ratio (Kmw) is a promising alternative chemical descriptor for surfactants instead of Kow, as it relates to partitioning into a critical cellular component and accounts for ionic interactions. Kmw can be determined by several experimental methods, but artificial phospholipid as dissolved unilamellar liposomes is considered the “gold standard”. Consistent Kmw values have been measured for a variety of surfactants using bilayer membranes coated on silica particles. Also promising computational approaches to calculate Kmw have been evaluated but require further calibration for surfactants. The overall aim of this study is to systematically evaluate three experimental methods and three computational approaches to determine Kmw for a diverse set of surfactants, covering nonionic, anionic, cationic, and zwitterionic structures. Based on this assessment, we aim to further establish confidence in the Kmw approach for different types of surfactants, provide guidance on the most cost-effective assay for specific surfactant classes, calibrate computation approaches, and make a next step towards standardization of validated test protocols. Here we present the Kmw measurements for 12 surfactants using three experimental methods, with benchmark dissolved unilamellar liposomes requiring dialysis systems, solid-supported lipid membranes (SSLM) which only require mild centrifugation, and HPLC columns with Immobilized Artificial Membrane coated silica (IAM-HPLC). The pros and cons of each experimental assay will be elaborated. Furthermore, the experimental data set allow for calibration of molecular computation methods, such as molecular dynamics and polyparameter LFER approaches. The data set provides relevant data to derive first tier estimates for surfactants on bioaccumulation and most likely also on baseline toxic potential, addressing ECHA’s 2023 Key Areas of Regulatory Challenge.

### 3.01.P-Th137 Scoping Key Sources of Uncertainty in Bioaccumulation Assessment: Biopartitioning and Biotransformation

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Bioaccumulation assessment is a critical endpoint of chemical hazard and risk assessment for ecological and human health. Bioaccumulation (B) assessment is challenging, with various metrics to consider, such as lab bioconcentration factors (BCFs), lab and field biomagnification factors (BMFs), and field bioaccumulation factors (BAFs), numerous regulatory criteria, and the natural diversity of biology and chemistry. Therefore, a weight-of-evidence approach for B assessment is often recommended in regulatory programmes.

An OECD Integrated Approach to Testing and Assessment (IATA) for Bioaccumulation was recently developed, by the Environment Agency (England), HESI and other key stakeholders. This “B IATA” provides guidance for B assessment and for addressing key sources of uncertainty in an efficient and scientifically defensible manner that reduces the need for animal

testing. The foundation of the B IATA is supported by generic 1-compartment physiologically-based toxicokinetic (PBK) models that can integrate measured *in vivo* and *in vitro* data as well as *in silico* data.

Fundamental to the bioaccumulation phenomenon in aquatic and air-breathing organisms are two underlying processes: biopartitioning and biotransformation. Traditionally octanol has been used as a surrogate for biopartitioning of neutral organic chemicals to various biological compartments, most notably adipose tissue (i.e., storage lipids). It is now recognized that octanol is not always a reasonable surrogate for biopartitioning for all chemicals, particularly for polar (neutral) and ionizable organic chemicals. This work presents a critical review for measuring biopartitioning to protein and membrane lipids, and estimation methods for these properties. This compliments the B IATA framework, which allows for the use of higher tiered biopartitioning data to replace “octanol as a surrogate for biopartitioning”. This work also presents a review of available methods for measuring (or estimating) biotransformation rates *in vivo*, from *in vitro* assays, and from *in silico* models. This review provides a starting point for developing IATAs specifically for addressing uncertainty in biopartitioning and biotransformation.

### **3.01.P-Th138 Regulatory Application of a Toxicokinetic-based Bioaccumulation Model**

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The bioaccumulation of active substances (a.s.) in organisms and their transfer through the food chain is a complex phenomenon involving several mechanisms, influenced by a wide range of biotic and abiotic variables. Although there has been significant progress in the consideration of bioaccumulation in the regulatory arena under global regulations, it is still quantified as a simple process driven mainly by lipophilicity (by considering the octanol–water partition coefficient;  $K_{OW}$ ). Typically, in addition to  $K_{OW}$ , the Bioconcentration Factor (BCF) in fish is used as the cut-off criterion for hazard assessment and for the evaluation of secondary poisoning to birds and mammals. Therefore, more accurate methods covering the complexity of this process are deemed necessary for addressing the potential concerns related to the increase in the concentration of a substance in organisms over time. Hence, we propose a bioaccumulation model (considering toxicokinetic parameters) to be applied in support of the risk assessment of chemicals under different regulations. The model presented in this work is already validated and calibrated with independent data (Alonso *et al.*, 2008) and is in consequence ready to use for regulatory applications. In this work, we investigate in detail the applicability and related challenges of this model for the “B” assessment under REACH Regulation (Annex XIII, 3.2.2) and for the refinement of secondary poisoning under Regulation 1107/2009 for Plant Protection Products. Additionally, we have described potential implementations of the model that would allow its application in other fields within the regulatory framework. Overall, it is concluded that the model can provide significant information to support the risk assessment of chemicals by reducing the uncertainty associated with the bioaccumulation process. The model can therefore be recognized as a versatile tool for regulatory applications involving bioaccumulation

### **3.01.P-Th139 Celebrating Some of the Many Scientific Contributions of Don Mackay: The Role of Chemical Fugacity and Activity in PBT, Exposure and Risk assessment**

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The hazard and risk from organic chemicals present in the environment are routinely evaluated using P (Persistence), B (Bioaccumulation) and T (Toxicity) data and criteria. We present a perspective on how models based on the equilibrium criteria of fugacity and chemical activity can contribute to PBT hazard evaluations as well as exposure and risk estimation, providing a consistent and integrated assessment process. The Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations have set out guidelines for evaluating PBT characteristics using bright-line criteria, however, use of these guidelines can lead to the misidentification of some chemicals. For example, the use of the octanol-water partition ratio ( $K_{OW}$ ) alone can misidentify chemicals with bioaccumulation potential in air-breathing species.

Overall persistence and chemical residence times have been advocated for because they are more representative of chemical degradation in the overall multi-media environment. Bioaccumulation is driven by toxicokinetic processes that can be estimated using fugacity-based models that treat uptake by respiration and diet, in single organisms or in food webs.

Biomagnification within food webs is of regulatory concern and is particularly important for air-breathing organisms because the atmospheric capacity for chemical is usually relatively lower compared to the corresponding aquatic chemical capacity. Compounds with a baseline narcosis toxic Mode of Action (MoA1) are believed to fall in the domain of a ‘narcotic fugacity’ (or activity) endpoint, in which a factor ‘N’ is defined as the fraction of saturation vapour pressure, solubility, or activity that causes onset of narcosis. Ranges of fugacities and activities corresponding to narcosis can be defined for specific species or regions thus enabling comparison of species sensitivity and regional or temporal trends with respect to exposure and risk. Multipliers for chemicals with more specific MoA can be considered.

We promote some of the scientific advancements that Don Mackay pioneered showing that chemical fugacity and activity provide a means to integrate hazard and risk information in the environment in simple and relatively easy-to-use mass balance multi-media models.

### 3.01.P-Th140 Mixture Toxicokinetic Assessment of Pharmaceuticals in *Daphnia magna* using Liquid Chromatography Tandem Mass Spectrometry Analysis

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Contaminants originating from anthropogenic activities are a source of environmental pollution. This includes pharmaceuticals that are released via various sources such as wastewater discharge from households, hospitals and manufacturing facilities. Pharmaceuticals are designed to target conserved biological pathways so the exposure of non-target organisms presents an environmental risk, necessitating a thorough examination of toxicokinetics (TK) to assess the potential for bioaccumulation. The work herein presents a liquid chromatography tandem mass spectrometry (LC-MS/MS) method capable of quantitatively measuring 90 pharmaceuticals, spanning various therapeutic classes (e.g., anticonvulsants, antidepressants, lipid regulators), at trace levels in *Daphnia magna*. The LC-MS/MS method was developed, optimised, and validated with acceptable linearity ( $R^2 \geq 0.98$ ) and repeatability ( $\leq 15\%$  RSD) across a dynamic range from low ng to  $\mu\text{g g}^{-1}$ . Optimisation involved comparison of different extraction solvents, volumes, lipid removal and SPE sorbents. Subsequently the validated method was applied to determine the internal concentration and TK profiles in *Daphnia magna* during 96-hour exposure experiments, which included a 48-hour exposure period, followed by a 48-hour depuration phase. Multiple sampling points enabled a temporal analysis to enhance understanding of uptake and elimination kinetics. Exposures were compared between individual pharmaceuticals and mixture-based experiments of 2, 5, 10 and 20 pharmaceuticals that were exposed simultaneously. The work aimed at exploring the potential of mixture exposures to reliably assess toxicokinetic parameters without the need for testing individual chemicals. This approach would reduce the number of animals required to experimentally determine bioconcentration or bioaccumulation during environmental risk assessments.

### 3.01.P-Th141 Integrated Approach for Testing and Assessment (IATA) for Bioaccumulation

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Bioaccumulation is the net result of competing rates of chemical uptake into, and elimination from an organism. Bioaccumulation assessment is an essential endpoint in national and international chemical regulatory programmes and treaties, e.g., the identification of Persistent, Bioaccumulative and Toxic (PBT) substances and Persistent Organic Pollutants (POPs). Bioaccumulation assessment is both a scientific and regulatory challenge due to the intricacy of the subject, the availability of reliable and relevant data, and lack of guidance in how data should be evaluated and weighted to inform decision-making. This process becomes more challenging considering variations in accepted data and the varying threshold criteria of different regulatory jurisdictions. A weight of evidence (WoE) approach is recommended by most established regulatory jurisdictions, such as REACH program in Europe, The Canadian Environmental Protection Act and the United Nations Stockholm Convention; however, there is no clear guidance for implementing a WoE approach for B assessment. Multiple lines of evidence (LoE) can be used to quantify, measure, and qualify the common metrics of bioconcentration factor (BCF), bioaccumulation factors (BAF), biomagnification factors (BMF) and trophic magnification factors (TMF). These LoE can comprise *in silico* predictions, combinations of *in vitro* and *in silico* data, e.g., *in vitro-in vivo* extrapolation (IVIVE), and laboratory or field data. Quantitative Structure Activity Relationship (QSAR) models for BCF and BAF, and biotransformation half-lives, as well as mechanistic bioaccumulation toxicokinetic (TK) models are established. Additionally, there are internationally recognised OECD test guidelines for the laboratory determination of *in vitro* fish biotransformation rates, BCFs, and BMFs. An OECD Integrated Approach for Testing and Assessment (IATA) for Bioaccumulation has been developed that encompasses the aforementioned LoE along with existing OECD testing guidance and WoE guiding principles to aid evaluators in the collection, generation, evaluation, and integration of multiple LoE for clear and transparent decision making in B assessment for aquatic and terrestrial environments. Three illustrative case studies representing both data poor and data rich chemicals are presented to illustrate the applicability of the IATA for B assessment.

### 3.01.P-Th142 Integrating In Vivo, In Vitro, and In Silico Approaches to Assess Chemical Toxicokinetics: A Cross-Species Comparative Analysis

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Limited availability of high-quality measured toxicokinetic (TK) data introduces uncertainty in hazard and risk assessments for various chemicals. Terminal elimination half-lives ( $HL_T$ ) are required in many contexts, including inter-species extrapolation of traditional toxicity data and *in vitro-in vivo* extrapolation (IVIVE) of bioactivity data obtained from New Approach Methods (NAMs). A key determinant of inter-species extrapolation is the differences in TK (e.g., biotransformation half-life,  $HL_B$ ) between species from different taxa. Allometric scaling and the use of uncertainty factors (e.g., "factor of 10") to extrapolate TK and toxicity data from one species to another are normally used. However, in most cases, these scaling factors are "arbitrarily" selected and may not be representative of the true uncertainty. The objective of this work is to apply bioinformatic tools to examine differences in  $HL_B$  and  $HL_T$  from various organisms (e.g., human, rat, fish) using different estimation methods including *in vivo*, *in vitro* and *in silico* data.

In vivo TK data and in vitro biotransformation rate data from hepatocyte, microsomal and hepatic S9 bioassays were obtained and critically evaluated using OECD testing guidelines and professional judgment to identify key sources of uncertainty in the data. OECD validated Quantitative Structure-Activity Relationships (QSAR) were used to predict  $HL_B$  and  $HL_T$  in mammals and fish. Allometric scaling based on body mass was applied to normalize the data and investigate TK differences between species. Variability in  $HL_B$  and  $HL_T$  between key species was scrutinized to test if commonly applied uncertainty factors for inter- and intra-species differences are supported by the data. There is general agreement in  $HL_B$  and  $HL_T$  estimates obtained from in vitro, in vivo and in silico methods for most chemicals supporting the advancement of non-animal methods for obtaining key TK parameters. For instance, based on a comparison of 218 chemicals the mean difference between body mass normalized rat and human in vivo  $HL_T$  is around 2 and an uncertainty factor of about 60 captures 95% of the variability in inter-species differences. This study supports the need for integrated and systematic testing methods for key TK processes to address the significant uncertainty that exists in TK data for thousands of chemicals and in the application of new and existing hazard data for human and ecological health assessment.

### **3.01.P-Th143 Contaminant Biomagnification in Humans: Interindividual Differences and the Role of the Gut Microbiome**

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The process that can lead to concentrations of an organic contaminant in an organism exceeding those in its diet is called biomagnification. It is a highly undesirable property of a chemical, as it allows it to become enriched in organisms at the top of a food chain, increasing the likelihood of having a detrimental effect on the health of those organisms. Although of obvious importance to humans, biomagnification has hardly ever been studied in humans, because the available methods are highly invasive. Here we applied an entirely non-invasive method that relies on the equilibrium sampling and chemical analysis of paired dietary and fecal samples to determine the thermodynamic limit to biomagnification ( $BMF_{lim}$ ) as well as the feces-based biomagnification factor ( $BMF_F$ ) for a selection of polychlorinated biphenyl congeners in humans. Over a period of five days, we collected diet and feces from five volunteers. The volunteers (i) shared the same diet, relatively high in animal lipids, to eliminate the confounding factor of dietary composition and (ii) recorded both dietary intake and fecal egestion rates. Using equilibration with silicone film, we determined the fugacity capacity of diet and fecal matter for PCBs and also the PCBs' fugacity in diet and feces. As the gut microbiome has a strong influence on the ability of the digestive system to absorb the diet, and dietary lipids in particular, high-throughput 16S RNA gene sequencing was used to characterize the gut microbial community of the volunteers immediately prior to the experiment and again at the end of the five days. The individuals differ widely (by as much as a factor of 3) in their biomagnification capabilities even though they shared the same diet. This is due to divergent dietary digestion efficiencies, which influenced both lipid assimilation efficiency (ranging from 98 to 99%) and fecal egestion rate  $G_F$  (ranging from 150 to 390 mL/day). The gut microbiome varied widely between individuals and experienced shifts after the change in diet. We will discuss in detail the role of age, sex, ethnicity, and gut microbiome in relation to the measured  $BMF_{lim}$  and  $BMF_F$ . Preliminary results indicate that the oldest volunteer (i.e., M58) had the highest lipid assimilation efficiency lip% (~99%) and  $BMF_{lim}$  (~70); while the third oldest person (M32) had the lowest  $BMF_{lim}$  (~25) due to a low digestion efficiency (lip% = ~98 %;  $G_F$  = 390 mL/day).

### **3.01.P-Th144 Monitoring freely dissolved concentrations of hydrophobic pollutants improves understanding of pollutant transport and bioaccumulation with clarity on sustainable management options**

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The development of equilibrium passive sampling methods has accelerated over the last decade and enabled measurements of ultra-low concentrations of bioaccumulative chemicals in the aquatic environment. Yet, use in the regulatory context has been limited due to the lack of precedent and solid demonstrations tied closely to regulatory needs. Remedial investigations of sites contaminated with legacy pollutants like polychlorinated biphenyls (PCBs) have traditionally focused on mapping sediment contamination to develop a site conceptual model and select remedy options. This sediment-focused approach often leads to an incomplete understanding of the impacts of ongoing inputs to the water column and over-estimation of potential effectiveness of sediment remediation. Here we demonstrate how measurements of surface water, sediment porewater, and gas-phase PCB concentrations using polyethylene passive samplers were able to provide the first accurate mass balance of freely dissolved PCBs in the Anacostia River in Washington DC. This mass balance enabled identification of a major tributary and contaminated sediment hot-spots as the sources having the most negative impact on the water body, while also demonstrating that the atmosphere served as a sink for PCBs from the surface water. By coupling the mass balance model with a food web model for the river, we were able to connect the media concentrations to bioaccumulation in fish and evaluate a range of management options. We demonstrate that sediment cleanup alone will not be able to achieve target reductions of PCBs in fish and dissolved concentrations from the problem tributary needs to be controlled. The framework presented here for passive sampling and interpretation can be used to provide robust and sustainable solutions for other river systems.

### **3.01.P-Th145 "Chasing Chemical Echoes: Unveiling Bioconcentration and Bioaccumulation of Legacy POPs and Emergent Compounds in the Waters of King George Island (Antarctica)"**

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The industrial advancements of the last century have significantly impacted Antarctic ecosystems through the release of Persistent Organic Pollutants (POPs) into the atmosphere. These persistent, semi-volatile pollutants possess the capacity to traverse considerable distances, reaching polar regions. They exhibit a propensity for bioaccumulation and biomagnification within trophic networks, inducing deleterious effects on the local biota. Scant attention has been devoted to comprehending these processes within antarctic water column with few studies previously published. A critical aspect lies in elucidating the complex interplay of the biological pump in the transport of COPs within water columns and trophic networks, underscoring the integral role of phytoplankton in these processes. The target compounds of the present study are Polybrominated Diphenyl Ethers (PBDEs), Polychlorinated Biphenyls (PCBs), and Organochlorine Pesticides (OCPs). We studied the occurrence of these compounds in seawater, phytoplankton and zooplankton. Analysis of biomagnification factors has revealed noteworthy patterns; specifically, higher LogKow congeners of PBDEs did not exhibit an elevated concentration across trophic levels, in contrast to PCBs, while OCPs displayed an intermediary behavior. The present work explores potential causes affecting bioaccumulation patterns and delves into the influence of potential PBDE and some OCP metabolism at the invertebrate level, explaining why their behavior deviates from hydrophobicity expectations. Additionally, the discussion encompasses an exploration of potential oceanographic factors influencing the environmental fate of these compounds. Satellitely measured primary productivity and upwelling oceanographic conditions emerge as pivotal elements, seemingly playing a significant role in the trophic transfer of these compounds. This nuanced analysis adds a layer of insight into the broader context of POPs dynamics in polar environments.

### **3.01.P-Th146 Occurrence, distribution, and bioaccumulation patterns of contaminants in coral reef systems**

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Coral reefs are exposed to multiple stressors caused by anthropogenic actions, which, combined with climate change, have resulted in drastic declines worldwide. The release of contaminants from different sources such as treated or untreated municipal, industrial, and agricultural wastes has been a major concern over the last decades. Yet, information on their concentration in such unique ecosystem is still scarce. Therefore, the present study aims to provide baseline knowledge on the distribution and concentration of organic contaminants (e.g., medicines, stimulants) and trace metals in the Red Sea coral reefs. Sediments, seawater, and reef fishes collected from three different areas with different levels of anthropogenic pressures (a total of 19 reefs) will be analysed to investigate the extent to which bioaccumulated concentrations of targeted compounds in fish can be predicted by concentrations in environmental compartments (water and sediments). We predict that the concentration of contaminants will increase according to the level of anthropogenic activities in the three different regions. Additionally, we expect to observe changes in the bioaccumulation patterns across three fish feeding traits (e.g., detritivores, corallivores, herbivores, carnivores). This study will contribute to provide scientific baseline information for future national environmental assessments and to guide ecotoxicology studies. It will also provide insight into cascading effects on associated species, underlining the potential health risk

### **3.01.P-Th147 Bioaccumulation Of Selected Pesticides In Freshwater Invertebrates**

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Several extraneous stressors threaten aquatic ecosystems nowadays. Some of these stressors are rising temperatures due to climate changes or adverse effects of emergent pollutants related to human activity. To fill gaps in knowledge necessary to predict the responses of freshwater biota to these stressors, we performed a mesocosms experiment focused on the effect of pesticides on freshwater invertebrates under different temperatures. Mesocosm is supposed to imitate small-standing waters. The mesocosms were inhabited by common macroinvertebrates and plankton taxa from different trophic levels. We further limited observations to six relevant pesticides and their metabolites. The pesticide solution with environmentally appropriate concentration was added to the systems once at the beginning of each experiment. During a four-month experiment in winter and a two-month experiment in summer, we collected water samples and determined the change in pesticide concentration in the systems over time. At the end of the experiment, all macroinvertebrates were analyzed for concentrations of not only pesticides but also their metabolites. As expected, different species bioaccumulated individual pesticides differently. However, terbuthylazine dominated all examined species, accompanied by its metabolite terbuthylazine-desethyl. On the contrary, propiconazole showed significant differences in bioaccumulation among species depending on their way of life. Mesocosms experiments were part of the project supported by the Czech Science Foundation (No. 20-16111S, "Understanding the effects of multiple stressors on freshwater biota: Will climate change alter the impact of chemical pollution?") and by the Ministry of Education, Youth and Sports of the Czech Republic – projects "CENAKVA" (LM2018099).



### 3.01.P-Th148 Bioaccumulation Dynamics of Ionizable Pharmaceuticals in Freshwater Crayfish

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Ionizable micropollutants are often bioaccumulative in aquatic biota. While pharmaceuticals are designed to exhibit high bioactivity at low doses in humans or animals, the dynamics of their bioaccumulation in invertebrates have been relatively understudied. Promoting the principles of the 3Rs (replacement, reduction, refinement) in animal welfare offers a promising avenue for encouraging innovation and aligning with European and international mandates aimed at reducing the use of vertebrates in research and regulatory practices. Freshwater crayfish play a prominent role in aquatic ecosystems and occupy significant niches within the trophic web. Notably, invasive crayfish species such as *Procambarus virginalis* and *Pacifastacus leniusculus* serve as excellent testing models, given their large body sizes and heightened sensitivity to environmental contaminants. This study seeks to expand upon recent mechanistic bioaccumulation modelling efforts that have traditionally focused on fish, guided by principles rooted in mammalian pharmacokinetics. In a series of 10-day bioconcentration experiments, comprising a 7-day uptake phase and a subsequent 3-day elimination phase, we monitored the concentration of diphenhydramine in crayfish hemolymph and whole body, while simultaneously calculating bioconcentration parameters. Both modelled and observed effects of the tested compound were rigorously assessed. The findings revealed the uptake and elimination kinetic parameters and the bioconcentration factor of model ionizables. Importantly, our research highlighted that water pH and dissociated compound quantity influenced the bioaccumulation dynamics. These bioconcentration experiments, driven by empirical data, shed light on the potential of crayfish as an invertebrate bioaccumulation model for ionizable pharmaceuticals, providing valuable insights for future studies in the ecotoxicology area. *The research was supported by the Czech Science Foundation (23-07274S) and by the Ministry of Education, Youth and Sports of the Czech Republic – project “CENAKVA” (LM2018099).*

### 3.01.P-Th149 Development of Meta-Analysis Techniques to Evaluate the Biomagnification Potential of Contaminants in Wildlife and Food-Webs For Environmental Risk Assessments

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There are many examples of chemicals that exhibit substantial variation in their estimated potential to biomagnify in organisms and food-webs, which often leads to uncertainty in how best to characterize them and make regulatory decisions for them. For example, field studies investigating the bioaccumulation of decabromodiphenyl ethane (DBDPE; CAS: 84852-53-9) in food-webs or wildlife have reported highly variable biomagnification factors (BMFs) and trophic magnification factors (TMFs) for DBDPE; consequently, regulatory decisions regarding the biomagnification potential of DBDPE have been inconclusive to date. Thus, we reviewed the literature for peer-reviewed studies reporting field-derived BMFs and/or TMFs for DBDPE with the objective to (i) develop a methodology using a weight of evidence approach and meta-analysis that can evaluate field-based biomagnification studies and (ii) apply it to DBDPE with the aim to inform regulatory decisions on whether DBDPE has biomagnification and/or trophic magnification potential. For the weight of evidence approach and meta-analysis, we developed several critical criteria with a scoring and categorization system to evaluate the quality and reliability in the reported BMFs or TMFs. For field studies with reliability scores above 50% (i.e., considered acceptable), one-sample t-tests indicated that the mean BMF of DBDPE was 0.56 (0.18 SE;  $t(18) = -0.23$ ;  $p > 0.05$ ) and the mean TMF of DBDPE was 0.95 (0.48 SE;  $t(7) = -0.098$ ;  $p = 0.92$ ) and thus not statistically different from 1. We also examined the log-linear relationship between reported BMFs and TMFs and their associated reliability score to evaluate if the quality of the study was related to the BMF or TMF. The reliability scores of the field studies marginally increased as the reported BMF ( $p < 0.05$ ;  $r^2 = 0.37$ ;  $F_{1,15} = 10.5$ ) or TMF ( $p = 0.36$ ,  $F_{1,11} = 0.85$ ,  $r^2 = -0.012$ ) in the study decreased. Typically, field studies with acceptable or reliable quality scores (i.e., > 50%) reported BMFs and TMFs that were less than or equal to 1, whereas field studies with unacceptable quality scores (i.e., < 50%) often reported BMFs and TMFs greater than 1. Ultimately, this weight of evidence approach and meta-analysis was effective for assessing the biomagnification potential of a super-hydrophobic substance like DBDPE and indicated that concentrations of DBDPE exhibit a limited potential to bioaccumulate and biomagnify in top predators and food-webs.

### 3.02.A Advances in Exposure Modelling to Inform Science-Based Environmental Solutions

#### 3.02.A.T-01 Quantification and mapping of tyre wear emissions: from EU regional analysis to global projections

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Tyre wear particle (TWP) emissions constitute one of the predominant sources of microplastic pollution in the environment. Numerous studies have endeavored to estimate annual national TWP emissions. However, a significant degree of variability exists in these estimations due to the diversity in the methodologies employed and the emission factors applied. The primary objectives of this study are to analyze the variability in the results obtained from different estimation techniques, and to provide a guidance for national estimates of TWP emissions. Our findings indicate substantial discrepancies in the per capita emissions estimated using the mass loss of tyres and emission factors across almost all Southern European countries and certain Northern European nations, with variances reaching up to 2 kg/y\*cap. In contrast, Western and Eastern European countries show minimal variation in results despite differing methodologies. Furthermore, extrapolating from the estimations,

we estimated annual TWP emissions of EU member states into various environmental compartments, including air, soil, sewers, and surface waters. Based on our research estimates, Germany, France, and Italy are considered the primary emitters of TWP, with annual emissions from each country exceeding 100,000 tons. It is imperative to highlight that a predominant portion of these emissions is deposited in soils adjacent to roadways. Despite the considerable overall emissions, only a limited fraction of these particles enters surface waters. Taking into account the variations in the coverage and efficiency of wastewater treatment plants, it is estimated that merely about 13,000 tons per year from these three countries make their way into surface. To predict annual TWP emissions for nations lacking specific data to estimate TWP emissions (e.g., annual mileage data), a multivariate linear regression model was developed that uses demographic variables as input. The model's results, including per capita and total TWP emissions for 57 countries across six continents, illustrate that Luxembourg leads in per capita TWP emissions with 3.56 kg/y\*cap, followed by the US with just over 3 kg/y\*cap. On a national scale, China and India, the two most populous nations globally, have the highest annual emissions of 1.5 million tons and 870,000 tons, respectively. The predictive model proposed here significantly simplifies the preliminary estimation process for national TWP emissions by lowering data needs.

### **3.02.A.T-02 Application of the Abrasion Coefficient as Measure for Tyre Wear and Microplastics Emission Modelling**

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Tyre Wear due to the friction between the tyre and the road contribute towards the emission of microplastics and other pollutants. Measuring the abrasion rate of tyres in mg per driven km will be an important aspect of the new EURO7 emission standards. Here we propose a novel method for quantifying Tyre Wear based on an abrasion coefficient in mg per J friction work determined from abrasion rate measurements. This is done by estimating the level of friction work energy that the tyres are subjected to during driving manoeuvres which are dependent on the characteristics of the road, the vehicle and driver behaviour.

The friction work can be estimated for track, road and lab scale abrasion rate measurements. The abrasion coefficient (mg/J) is then estimated as the measured abrasion rate (mg/km) divided by level of friction per km track (J/km), in the case of track measurements. Such an abrasion coefficient is a much more generic measure for abrasion compared to the more commonly derived abrasion rates (mg/km) which are specific to a road type (e.g. highway versus urban roads), vehicle type and the specific manoeuvres performed. The model code is available from <https://github.com/rivm-syso/tyre-friction-abrassion-emission>.

The Tyre Wear Abrasion Model could allow calculating the effects of different future scenarios, such as transition to a more electrified fleet or towards heavier vehicles (i.e. SUVs). This allows for more targeted policy advice in view of the goals for microplastic pollution reduction of 30% by 2030 defined in the EU Action Plan "Towards Zero Pollution for Air, Water and Soil". In this work the Tyre Abrasion Model is calibrated based on ADAC abrasion measurements as well as novel measurements from the LEON-T project. The utilisation of the model is illustrated for Tyre Wear as part of microplastics emissions in the Netherlands. This is done to compare Tyre Wear to other microplastics emissions in order to discuss their overall contribution towards (micro)plastic pollution.

### **3.02.A.T-03 Mapping emerging contaminants in sewage sludge treatments: Occurrence, degradation, and fate**

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The use of sewage sludge (SS) as fertilizers is undergoing increasing scrutiny because of potential risks from the increasing amounts of pollution from hazardous metals and organic compounds entering wastewater. Among the hazardous organic compounds (HOCs), per- and polyfluoroalkyl substances (PFAS), a complex group of synthetic chemicals that have been used in consumer products, and organophosphate flame retardant (OPFRs), a variety of consumer products used as flame retardants and plasticizers, are of rising concern because of their long-lasting persistence in the environment and food products with harmful health effects in humans and animals. Improvements in SS treatment options that abate the presence of HOCs are needed to mitigate this threat to an expanding circular economy. Thermal treatments are becoming attractive in this context, also because they can co-deliver useful energy and materials. However, there is limited information on the efficiency by which they can remove toxic pollutants. This study provides an accurate mapping of the fate of various pollutants (PFAS, OPFRs, BPA and hazardous metals) through different treatment steps. This study compares conventional and thermal treatments of SS by integrating SS sampling from wastewater plants in Norway of 61 HOCs (PFAS and OPFRs) and 12 hazardous metals and laboratory analysis with detailed process simulations through Aspen Plus. The scenarios are based on individual (or combinations of) thermal/non-thermal treatments such as anaerobic digestion, de-watering, stabilization, drying, pyrolysis, incineration, and valorisation of co-products (biogas upgrading, heat and power cogeneration, biochar application to soils). The results show that conventional SS treatment leaves between 24 and 40% of OPFRs unabated, while almost no degradation occurs for PFAS. Thermal treatments can degrade more than 93% of OPFRs and 95% of measured PFAS (with the rest released to water bodies via effluents). The different treatment options vary how HMs are emitted across environmental

compartments. A rigorous statistical approach is secured by a large data sampling and the implementation of a Monte-Carlo uncertainty. Overall, this study increases the availability of empirical data for removal efficiency of different sludge treatment options with process modelling. This work serves as a valuable resource on process designs for informed science-based solution for the treatment of waste containing HOCs.

### **3.02.A.T-04 Assessment of Wood Preservative Emission Scenarios**

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The Organisation for Economic Co-operation and Development (OECD) Emission Scenario Document (ESD) for Wood Preservatives provides methods to estimate copper release from treated wood for various scenarios including treated sheet pilings in a flowing waterway. In this research, ESD equations for copper release from treated sheet pilings were reviewed and compared to basic mass balance equations used in surface water quality modeling to better understand their theoretical basis. A screening-level numerical model, similar to the Tableau Input Coupled Kinetics Equilibrium Transport Unit World Model (TICKET-UWM), was developed to assess the transport, fate, and effects of metals in rivers, streams, and other flowing waters. This model, called TICKET-2D, was used to quantify copper concentrations in the water column and sediment resulting from release from sheet pilings.

Analytical solute mass transport equations derived from plug flow systems, which are typically used to assess chemical fate and transport in rivers and streams, were functionally equivalent to the ESD equations. Therefore, the chemical behavior in the ESD generalized flowing waterway is best described using equations/models for rivers and streams on the basis of system geometry and mixing characteristics.

TICKET-2D considers the same chemical and physical processes as TICKET-UWM. It consists of a basic two-cell unit with an overlying water cell and a sediment cell. This unit repeats in the direction of flow to simulate flowing systems like rivers and streams. TICKET-2D verification simulations accurately reproduced output from the ESD equations when run with identical input parameters and specified  $K_D$  values. Calculations/simulations made with ESD equations and the TICKET-2D model demonstrate that the critical area for use classification is sediment since this is where PEC values exceeded PNEC values for simulations with long term copper emission rates.

### **3.02.A.T-05 Model Development for Assessing the Impact of Accidental Radioactive Releases into the Meuse River-Campine Canal**

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Emergency responses to accidental releases of hazardous materials, including radionuclides into aquatic environments require site-specific information. While monitoring systems can be helpful in identifying possible hazards, simulated data—such as the arrival of a potential plume—will be helpful in preventing exposure to radiation. In this context, we study the aquatic systems of the Meuse River and its connected canal network (Campine Canal) that flows across Belgium. Where the river contains not one but two (Chooz and Tihange) nuclear power plants (NPP) and the downstream flow across both power plants runs through many major Belgian cities, including Antwerp, and serves as primary source of drinking water in this region. In this study, the one-dimensional coupled shallow water and transport equation is solved for radionuclide concentration using the discontinuous Galerkin method in the SLIM modelling framework, where the model is applied for hypothetical accidental scenarios for both the NPPs. The scenarios were selected in reference to past nuclear accidents, where it was seen that the primary contamination was due to atmospheric releases with two dominant radionuclides: one is a long-lived  $^{137}\text{Cs}$  and the other is a short-lived  $^{131}\text{I}$ . Therefore, the scenarios selected are for atmosphere deposition considering varying wind speeds, as well as two river discharge scenarios for both radionuclides. In general, the simulated results show that, among the network of canals, the movement of radionuclides is slower in the Albert Canal (largest canal in the Campine network) compared to others, whereas the deposited plume travels relatively faster in the Meuse River. In fact, the movement is so slow that, in some scenarios, even if the distance through the Albert Canal is shorter, the radioactive plume reaches Antwerp earlier through other smaller Campine Canals. While the radioactivity level of  $^{131}\text{I}$  in these smaller canals, during high flow conditions in the Meuse River becomes considerably low after up to 3 weeks of release, as opposed to the levels in the Albert Canal, which remain rather high. Hence, even though the arrival of radionuclide is slower in later parts of canal and preventive measures can be taken, the impact on drinking water in the Meuse River is immediate. Therefore, we would also present the ingestion doses for the general public for the different accidental scenarios.

### **3.02.B Advances in Exposure Modelling to Inform Science-Based Environmental Solutions**

#### **3.02.B.T-01 Antibiotics Pollution in the Three Gorges Reservoir Area: Current Status and Risk Analysis**

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The extensive use of antibiotics in human and veterinary medicine leads to environmental concerns, particularly their prevalence in ecosystems. To address this, the MARINA-antibiotics model, integrated with the SWAT tool for runoff, soil

erosion, and water flow, was applied to assess antibiotic pollution in China's Three Gorges Reservoir Area (TGRA). This approach dynamically simulates the monthly fate of 30 antibiotics. The study focused on the TGRA river basin, estimating the load and concentrations of antibiotics in water and sediment. It factored in antibiotic concentrations in human and animal waste, wastewater treatment efficiency, population sizes, and manure management. The research also assessed the potential risks of antimicrobial resistance from these antibiotics.

Our findings indicate substantial spatial and temporal variations in antibiotic pollution within the TGRA, largely influenced by population distribution and animal farming activities, as well as fluctuations in streamflow. In the TGRA region, the total estimated antibiotics load was approximately 593.4 tons, with 84% originating from human sources and the remaining 16% from livestock production. Regarding the pathway of antibiotic transmission from land to rivers, point sources accounted for 99.38% of the load, while diffuse sources contributed a minor 0.62%. Notably, Oxytetracycline (OTC), Sulfachloropyridazine (SCP), and Ofloxacin (OFX) emerged as the most prevalent antibiotics, with respective loads of 82, 73, and 65 tons. The overall antibiotic loading density in the area was calculated to be 11.39 kg/km<sup>2</sup>.

In our study of the fate of antibiotics in river systems, it was found that approximately 70% of these antibiotics eventually settle into the sediment, while 30% dissolve in the water.

Furthermore, the concentrations of antibiotics in water as predicted by our model were generally within an order of magnitude of the actual measured values, confirming the model's accuracy. Additionally, our study revealed a seasonal pattern in antibiotic contamination, noting increased levels during the dry season as compared to the wet season. Furthermore, an antibiotic risk assessment revealed that 6 out of the 45 river subbasins studied are at risk of antimicrobial resistance. These findings underscore the urgent need for effective management strategies to mitigate the environmental impact of antibiotic pollution and its associated risks in the TGRA and similar ecosystems.

### **3.02.B.T-02 Mapping the Risk of Ciprofloxacin in European Water Bodies: Incorporating the Impact of Bioavailability** *Qiyun Zhang, Ph.D. candidate<sup>1</sup>, Kristof Demeestere<sup>2</sup> and Karel De Schampelaere<sup>1</sup>, (1)Ghent University - GhEnToxLab, Belgium, (2)Ghent University (UGent), Belgium*

The fluoroquinolone ciprofloxacin (CIP) is a widely prescribed antibiotic applied in human and veterinary health care. With its production and application, the compound has been detected in surface waters worldwide, which may lead to unwanted ecotoxicological consequences such as the development of antimicrobial resistance (AMR). On the other hand, recent studies have shown that the ecotoxicity and bioavailability of CIP is affected by water chemistry factors, namely, environmental pH and the dissolved organic carbon (DOC). Therefore, a comprehensive environmental risk assessment (ERA) for CIP needs to encompass the interplay between CIP and environmental conditions.

This study incorporated bioavailability into the ERA of CIP. With a CIP bioavailability model, the sensitivity of European water bodies to CIP, expressed by an environmental prediction no-effect concentration (PNEC<sub>ECO</sub>), was estimated under four simulation scenarios. They are (1) the conventional scenario (no bioavailability incorporated), (2) the worst-case scenario (pH affects bioavailability, but DOC has no effect), (3) the average scenario (both pH and DOC affect bioavailability, weak CIP-DOC interaction), and (4) the best-case scenario (both pH and DOC affects bioavailability, strong CIP-DOC interaction). Probabilistic risk assessments were performed based on Monte Carlo simulation using CIP occurrence data and PNEC<sub>ECO</sub> obtained in different scenarios. Probability of risk ( $P_{risk}$ ), uncertain risk ( $P_{uncertain\ risk}$ ), and safe ( $P_{safe}$ ) were assessed for Europe as a whole and for ten nations specifically.

Results indicate that the PNEC<sub>ECO</sub> had a high regional variability. Western Austria, northern and central Italy, southern Greece, and Norway appeared to be more susceptible to CIP. On the European scale,  $P_{risk}$  was between 9.8% (best-case) and 21.7% (conventional) across different scenarios, showing a clear influence caused by DOC. The country specific  $P_{risk}$  also presented high variations. The  $P_{risk}$  estimated for Spain and the Netherlands was below 10% in all scenarios, and that for France was 13-21%. The influences of pH and DOC were greater on the  $P_{risk}$  estimated for Germany and Portugal, which ranged from 32-67% and from 4-99%, respectively. Austria, Croatia, Czech, Hungary, and Slovakia observed probability of uncertain risk between 21% and 50%, while the incorporation of bioavailability could reduce uncertainties. Overall, the inclusion of water chemistry factors enables a more accurate ERA

### **3.02.B.T-03 Nano- and microplastic particles as vectors of exposure for plastic additive chemicals: Exploring the human health implications through the use of a multimedia food web model**

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Nano- and microplastic particles (NMPs) may present a hazard in various ways, including a direct biophysical response to physical particle properties (e.g. size and shape), or as vectors for chemical exposure (e.g. plastic additive chemicals and/or those that might become sorbed to the particles from the surrounding environment). Evaluating risk requires reliable and relevant characterization of both exposure and hazard. The leaching of chemicals from NMPs has been identified as a potential exposure pathway in studies reporting effects associated with both in vivo and in vitro test systems. However, it remains unclear whether this exposure pathway is relevant to human health under environmentally relevant

conditions. Previously, the bioaccumulation food web model (ACC-HUMAN), has been used to estimate human exposure to hydrophobic organic chemicals as a result of consuming contaminated food and beverages obtained from the terrestrial and marine environments. Here, we describe a modification of the steady-state version of the ACC-HUMAN model to include dietary exposure to NMP containing either accumulated chemicals from the surrounding environment or chemical additives embedded in the plastic. Chemical transfer to the organism is described using a two-film resistance equation assuming spherical particles of different sizes. Various scenarios are presented, including the ingestion and subsequent bioaccumulation of chemicals associated with NMPs in terrestrial and marine food webs, with tissue concentrations accumulated in humans compared against a point of departure (POD), assumed to be 1 for a suite of hypothetical chemicals with varying physicochemical properties. Results suggest that exposure to NMPs with an inclusion level of plastic additive chemicals of 5% wt/wt with varying physicochemical properties needed to exceed a risk characterization ratio (RCR) of 1, would be significantly greater than current estimates of exposure to NMPs and subsequent leaching of plastic additive chemicals. The model can, thus, provide the basis for enabling a tiered evaluation of the relative human health risks posed by exposure to NMPs with varying levels of plastic additive chemicals.

### **3.02.B.T-04 Exposure Modelling Approaches to Support Environmental Decision-Making in Multiple Contexts: A Review of Select Case Examples at the U.S. EPA**

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Currently, jurisdictions and individuals are challenged to consider the full range of information required to manage and use chemicals in a way that protects and promotes public health. Research to apply and demonstrate approaches that leverage disparate information by combining both mechanistic and data-driven modeling to characterize human exposure to environmental stressors is critical for credible decision-making. For example, there are persistent environmental contaminants that are contributing to many human health outcomes, including lead (Pb) and perfluoroalkyl substances (PFAS). For Pb, much is known about its sources, and it has largely been eliminated from production of consumer goods in the U.S., but historical contamination still poses a human exposure risk. In contrast, despite some phase-out of some legacy PFAS, alternate chemicals within the same class of compounds are being introduced into product streams. We are conducting applied research on these two high priority chemicals using the latest modeling techniques to inform decisions at the national, regional, and local scale. EPA's Stochastic Human Exposure and Dose Simulation model for Pb (SHEDS-Pb) — a probabilistic, mechanistic human exposure model combined with a Pb deterministic biokinetic and uptake model — was developed and applied to estimate aggregate children's exposures and blood lead levels (BLL) from drinking water, dust, and soil to support EPA regulatory Pb decisions. On a regional level, we developed an advanced geospatial statistical approach using children's BLL to identify high Pb exposure locations at the census tract scale for two U.S. states. The resolution of results at this scale can help the most impacted communities target Pb actions for public health protection. For PFAS, limited existing fish tissue PFAS occurrence data, publicly available geospatial data, and census information were used to develop predictive models to identify potential areas of PFAS contamination. These findings are informing additional fish sampling in the Northwestern U.S. that will help EPA Regional partners better focus efforts to reduce human exposure to PFAS in impacted communities. These examples demonstrate our commitment to using state-of-the-science information and exposure tools to: characterize the scope and magnitude of the most important environmental health problems; develop new information to fill the most critical gaps; and advance methods and tools to inform public policy.

### **3.02.B.T-05 Power to the (usage) data – opportunities and limitations of model-based emission estimation for environmental exposure assessment of chemicals**

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With increasing numbers of chemicals used in modern society, assessing human and environmental exposure to them is becoming increasingly difficult. For example, measuring all >26,000 chemicals registered under REACH seems unrealistic and would be incredibly expensive. Currently, exposure assessment relies predominantly on empirical monitoring studies and is therefore per definition (1) retrospective and (2) limited in terms of the chemical spectrum covered. We argue that estimating emissions of chemicals based on production and usage data could offer a more efficient and systematic approach to tackle this issue. To illustrate the approach, a case study was conducted in the city of Nijmegen (NL) to estimate the emissions of active pharmaceutical ingredients (APIs) to wastewater. Input data included prescription data and hospital usage data on local level as well as over-the-counter sales on national level. Various routes of administration were considered and monthly emission loads to the wastewater treatment plant influent were calculated. The results suggest that model-based emission estimation on a city-level is feasible and in good agreement with wastewater measurements obtained via passive sampling. Results highlight the need to include excretion fractions in the conceptual framework of emission estimation but suggest that the choice of an appropriate excretion fraction has a substantial impact on the resulting model performance. Overall, model-based emission estimation could represent a complementary tool to environmental monitoring, particularly for compounds for which emission sources and emission pathways to the environment are known. Data availability and accessibility currently represent a major bottleneck for applying model-based emission estimation to other groups of chemicals of emerging concern beyond APIs.

### **3.02.P Advances in Exposure Modelling to Inform Science-Based Environmental Solutions**

### 3.02.P-We116 A parametrized and regionalized Life Cycle Inventory Model to assess Tire and Road Wear Particles emissions

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Tire and Road Wear Particles (TRWPs), comprising degraded rubber, minerals, and road dust, are a significant global source of microplastics, estimated at 5.9 million tons in 2017. Recognizing the potential hazards to ecosystems and human health, this research addresses the limitations of current TRWP emission estimates. Indeed, current methodologies relying on emission factors (EFs) lack geographical applicability and often overlook critical parameters such as tire type, vehicle characteristics, road surface and driving styles. This study aims to improve the TRWP emissions inventory by incorporating these parameters, yielding context-specificity and reliable estimates. To calculate TRWP total emissions a bottom-up approach which integrates regional traffic activity rates with vehicle-specific EFs is employed. Initially, default EF values for six passenger and freight vehicles, distinguishing between large (10 µm to 500 µm) and fine (<10 µm) particle, were derived from an extensive literature review. Then, travelled vehicle-kilometers for the entire vehicle fleet were retrieved from international traffic databases. Finally, key parameters influencing tire wear, identified through a comprehensive review, were incorporated into the model as correcting multiplier factors for the default EFs. In addition, three archetypes, representing regional variations, were introduced. With 18 vehicle-specific default EFs and 8 key parameters, TRWP emissions were calculated for 34 countries. A sensitivity analysis under urban driving conditions emphasizes the relative impacts of influencing parameters, aiding prioritization for future investigations. Using default EFs, a comparison of TRWP total emissions with prior estimates demonstrates the model's reliability, with relative gaps of 8 to 34%. Corrected EFs reveal significant changes in emissions according to considered parameters, emphasizing potential underestimations in prior inventories. This parametrized and regionalized model offers a comprehensive understanding of global TRWP emissions, capturing diverse scenarios. Acknowledging data limitations regarding influencing parameters, the model is designed for continuous refinement as additional data becomes available. The results of this model can be used in future Life Cycle Inventories and Life Cycle Impact Assessments of TRWPs, especially to shed light on the magnitude of their impacts in comparison with the rest of the impacts associated with road transport.

### 3.02.P-We117 EXTRAPOLATION OF AIR RELEASE RATES BETWEEN DIFFERENT SUBSTANCES BEING USED UNDER SIMILAR CONDITIONS

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In any quantitative environmental risk assessment on the use of a chemical, the determination of release rates to air are key in achieving realistic exposure estimations. While release rates are typically available for volatile substances, useful information is scarce or lacking for structurally related semi- and non-volatile substances.

Therefore, we are proposing a simple method to extrapolate air release rates between chemicals with different vapour pressures and solubilities being used under similar conditions, based on evaporation theory. The method utilizes the vapour pressure, activity coefficient and air diffusion coefficient to extrapolate air release rates between chemicals. The extrapolation method is applicable to release from liquids and solids, but not to release from spray uses or uses that mainly give rise to airborne particles.

This poster describes the extrapolation method in more detail and its specific application to petroleum products as an example. This method can potentially be easily implemented in the REACH Environmental Release Category (ERC) concept to result in more realistic environmental risk assessments.

### 3.02.P-We118 Improving release estimates for the use of REACH registered petroleum and petrochemical-borne substances

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In any quantitative environmental risk assessment on the use of a chemical, the determination of operational conditions (OCs), risk management measures (RMMs), and resulting local and regional release rates are key in achieving realistic exposure estimations. In the absence of specific data, release rates to air, water, soil and waste can be estimated by applying predefined release fractions (RF) on the local and regional use tonnage (for example, in kg/d). In REACH Guidance, default RFs are available for 25 generic use patterns (or Environmental Release Categories (ERC) in REACH terms). Industry sector groups (chemical manufacturers and their downstream customer industries) can further refine the ERC for each identified use of their chemicals into specific Environmental Release Categories (spERC), which describe the expected good practice conditions of use, and the corresponding RFs.

This poster describes recent collaborative efforts between the European Solvents Industry Group (ESIG), the European Solvents Downstream Users Group (ESVOC) and Concawe, the research association of European Fuel Manufacturers, to refine and where needed, develop additional spERCs that cover the uses of a wide range of petroleum derived substances: traditional solvents as well as complex substances whose precise composition is unknown or can only be partially characterized. Examples of such complex substances include gasoils, naphthas, mineral oils, white spirits, fuel oils, lubricants and refined base oils.

To refine and develop these spERCs, information from publicly available technical and scientific reports (for example, Emission Scenario Documents) as well as expert knowledge available at the manufacturers and downstream users are leveraged. Where feasible, sub-SpERCs supporting water solubility and vapour pressure dependent RFs are defined to allow further refinement of release estimates for the diverse range of substance properties. In the absence of specific data, generic operational conditions and RFs as defined in the REACH Guidance ERCs are adopted.

### **3.02.P-We119 Estimating Marine Chemical Emissions from Discharges of Sewage Treatment Plants into Freshwater Rivers**

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Changes in demographics are leading to greater urbanization and higher coastal populations which in turn is increasing the need to better understand chemical loading into coastal and marine environments. Many Home and Personal Care Products (HPCPs) are disposed of down the drain by the consumer, whereupon the ingredients typically are released into freshwater rivers having passed through upstream sewage treatment plants (STPs). Further transport to the marine environment can take place. However, there is a currently paucity of approaches to adequately estimate the extent of this emission route into the environment. Following on from previous work from our group estimating chemical mass discharged directly into marine environments, here we present an approach which utilizes data on hydrologic travel distance/time for inland STPs combined with estimated aquatic half-lives to estimate emissions from upstream STPs into rivers flowing to coastal areas. We have developed a spatially explicit fate and transport model to estimate emissions into the marine environment across 88 countries. This global model utilizes the HydroAtlas river dataset and covers 44,000 STPs in 34 countries which discharge into both freshwater and coastal waters directly. Ingredient mass discharged from freshwater STPs was estimated using hydrologic routing from the STP and estimated aquatic half-lives to the coastal discharge location. Discharged mass and percent of mass reaching the coast can then be calculated. Resulting information can be used to refine emission estimates of ingredients into marine environments based on population demographics, disposal scenarios, and STP hydrologic distance to the coast. Results can also be used to inform more refined marine exposure modelling for risk assessment purposes.

### **3.02.P-We120 European Monitoring Data Reveal Temporally Extended Pesticide Occurrence**

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Pesticides infiltrate aquatic ecosystems via various pathways, presenting substantial threats to non-target aquatic organisms. Traditionally, the evaluation of these risks has been limited to either acute or chronic assessments. However, it is imperative to advance our understanding of pesticide exposure patterns in aquatic environments and the profound impacts of pesticide applications and environmental dynamics. This knowledge is pivotal in establishing and verifying assumptions in ecotoxicological testing protocols. In this context, the utilization of comprehensive, large-scale monitoring data emerges as a transformative approach. This approach offers an expansive foundation for assessing aquatic exposure by examining sequential pesticide concentrations. This novel perspective will not only enhance our grasp of the dynamics of pesticide exposure in aquatic ecosystems but also contribute significantly to the development of ecotoxicological methodologies and the safeguarding of non-target aquatic species.

We investigated the likelihood of pesticides reappearing in European streams over medium-term (4 - 7 days) and long-term (8 - 30 days) periods, meaning their tendency to remain present at quantifiable levels for periods beyond the typical 96-hour (4-day) exposure duration, reflecting the minimum duration of chronic ecotoxicity testing. We collected and analyzed publicly available data on pesticide levels in European streams to establish probabilities of reoccurrence (POR) for approximately 360 different pesticides, ranging from less than 1% to 100%. By comparing medium-term and long-term probabilities of reoccurrence, we identified three categories that describe most pesticides: (1) occasionally, (2) repeatedly, and (3) continuously occurring substances. Fungicides were the most common among repeatedly occurring substances, while neonicotinoid insecticides and legacy compounds were found to continuously occur. The results of this study challenge the current understanding of how we assess the persistence and impact of pesticides and emphasize that substance-specific factors, such as physico-chemical properties, application recommendations, and regulations, influence how pesticides affect aquatic environments. This study underscores the need to evaluate pesticide exposure by considering consecutive concentrations, enhancing our understanding of the real exposure risks to aquatic ecosystems on a substance-by-substance basis.

### **3.02.P-We121 Modeling Global Environmental Fate of Short-, Medium- and Long-Chain Chlorinated Paraffins and Quantifying Global Source-Receptor Relationships for Remote Regions**

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The long-term and widespread use of chlorinated paraffins (CPs), including short-, medium- and long-chain CPs (SCCPs, MCCPs and LCCPs), has resulted in extensive emissions and subsequent pervasive presence in the environment and the exposure of human and wildlife populations. Adverse effects of CP emissions may not only manifest in local source regions, but may also occur in remote regions, if CPs have the potential for long-range transport in atmosphere and oceans. However, a

comprehensive understanding of the global spatiotemporal distribution of SCCPs, MCCPs and LCCPs is still lacking, which hinders us from quantifying the relative emission contributions of different source regions to the contamination of remote regions. Here, we apply the BETR-Global model with global emission estimates to simulate the global environmental fate of SCCPs, MCCPs and LCCPs. By feeding estimated emissions in East Asia, South Asia, Europe and North America separately into the model, we estimate their contributions to the environmental concentrations in three remote regions, namely the Arctic, Antarctica and the Tibetan Plateau. Through this work, we aim to: (i) identify the most CP contaminated regions globally and unveil the spatial and temporal distribution patterns in these regions; (ii) examine the temporal trends of environmental occurrence in the three remote regions, and how CP homologue groups fractionate and redistribute due to their varying potential for long-range transport; (iii) determine the regions contributing the most to CP contamination in remote areas, and explore how these contributions evolve in time and differ between SCCPs, MCCPs and LCCPs. This work not only advances a holistic view of the global environmental fate and transport of CPs, but also quantitatively reveals how remote environments respond to the evolving use and emission of CPs in source regions. Such global source-receptor relationships are crucial for evaluating the effectiveness of potential global and regional CP emission-reduction strategies.

### **3.02.P-We122 Using fugacity model to explore transport dynamics of polychlorinated biphenyls in coastal environments**

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Polychlorinated biphenyls (PCBs) are persistent organic pollutants that have affected the human living environment. Although PCBs have been banned in Taiwan, in recent years PCBs are still detected in Taiwan's coastal sediments and organisms including fish, shellfish and, mud shrimps. The aim of this study is to explore the spatial and temporal changes of PCBs in coastal environments based on the fugacity model. We construct a Level III fugacity model to describe the fate and transport of PCBs in coastal area near the industrial park to estimate the fugacities based on the PCBs measurements. Then, the estimated fugacities are used as the initial values in the Level IV fugacity model which describe the transport dynamics in the southward current scenario to simulate the time-course concentrations in two scenarios with emission sources and without emission sources for 3 months. Results show that PCBs are still released into the environment with estimated emission rate of 0.001 mol hr<sup>-1</sup>. Using the fugacity models could help to understand the fate and transport of PCBs and inform the dynamics of PCBs in coastal environment. In the future, other media could be added into this model that can also be further used to explore the effect of other parameters, providing implications on the coastal transport dynamics of PCBs for environmental planning and management.

### **3.02.P-We123 Modelling Fate and Transport of Pharmaceutical Active Ingredients in a Swedish Lake Receiving Wastewater Effluents**

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Active pharmaceutical ingredients (APIs) from human pharmaceuticals are continuously emitted into the aquatic environment from effluents of wastewater treatment plants. While most APIs are emitted in very low concentrations, many are persistent and can accumulate in aquatic environments, and some are bioactive at very low concentrations. In this study, we present predicted environmental concentrations of 503 prescription APIs in Lake Ekoln, a sub-basin of Sweden's third largest lake, Mälaren. Our model considers the annual consumption of APIs, human metabolization rates, removal at the wastewater treatment plant, as well as biodegradation and mass transfer in the lake. The methodology included the following steps: identification of prescribed defined daily doses, active ingredient concentrations and structures; calculation of the physico-chemical properties of the compounds; calculation of emission into wastewater of each individual API; calculation of degradation and partitioning in wastewater treatment plant; and three-dimensional hydrodynamic modelling of API concentrations in the lake, accounting for biodegradation. The model was validated using measured concentrations in wastewater effluent and in the lake. The model demonstrates that even if a chemical is continuously emitted at a constant rate, the concentration in the lake varies by time, location, depth, and biodegradation rate, with the largest deviations seen for chemicals with short half-lives. For this lake, using the middle of the lake as a reference point, the maximum factors of potential differences stem from location, followed by depth, time, and then biodegradation. The model provided an accurate prediction of API concentrations in the lake, with 85% of the API median modeled values being within a factor of 10 from the measurements. One of the main conclusions from this work is that the concentration in a recipient can be modeled with a high level of accuracy directly from wastewater effluent concentrations. Thus, monitoring of recipients could, at least in part, be performed directly from samples of wastewater effluents. Finally, the accuracy of the model clearly demonstrates that it is possible to provide useful predictions on environmental concentrations when precise data are available on the consumption and emission of a chemical.

### **3.02.P-We124 Plastic Fate and Transport in Rivers: A Holistic Micro- / Macroplastic Perspective**

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Over the last years micro- and macroplastic (or plastic debris) have been increasingly monitored providing a more and more detailed grid of sampling data. However, sampling data only provides spot information while the general desire would be continuous exposure data across catchments, rivers, or continents.



Here, we present a modeling approach for micro- and macroplastics in rivers and lakes on a country-scale. The model is based on existing small-scale modeling approaches and is parameterized using available monitoring or fate data and is coupled to a high-resolution release model. The model is able to predict concentrations of micro- and macroplastics throughout a whole country and was first implemented for Switzerland. Our work aimed to compare dominating processes for micro- and macroplastics in order to increase our process understanding and derive exposure maps. In detail, we found that fate and transport are different for both size classes. On a catchment scale (e. g. the Rhine catchment in Switzerland) we observed about 45% and 98% retention for microplastic and macroplastics respectively. Moreover, we estimated that the accumulation of microplastics might be concentrated in lakes, while macroplastic accumulation, i. e. through beaching at river banks, is widely spread throughout the catchment. Also, macroplastic pollution in rivers and at river banks was found to be highly related to emission sites due to fast retention processes (i.e. beaching). In contrast, microplastic pollution was predicted to be spread over longer distances through water bodies.

Finally, understanding the fate and transport will help to decide on mitigation strategies in order to decrease plastic pollution and the potential risk to flora and fauna. Moreover, using the presented processes understanding can be applied to estimate emission data based on plastic concentration measurements in rivers.

### **3.02.P-We125 UTOPIA: Advancing Microplastic Understanding Through Process-Based Mass-Balance Modeling**

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Plastic pollution is persistent and expands through all environmental compartments globally, however, the threat posed by plastic in the environment is still poorly characterized. Uncertainties about sources, transport, transformation and removal processes, and the properties of the plastic pollution itself still prevail. Simultaneously, plastic contributes to a complex profile of particulate pollution, characterized by a diverse range of composition, size, and shape, accompanied by a heterogeneous mixture of chemicals.

Inspired by evaluative unit world models for chemicals, here we present an open-source process-based mass-balance unit world model that provides a platform to synthesize knowledge about plastic pollution as a function of its measurable intrinsic properties.

Our modelling platform includes a reference set of equations to simulate transformation and transport plastic processes across a variety of soil, water, sediment and air compartments, as well a generic database of microplastic properties. Exposure indicators such as overall persistence (POV), characteristic travel distance (CTD) and transfer efficiency (TE), are derived enabling scenario analysis in comparative assessments of alternative plastic materials.

Our platform aims at providing a reference modeling platform for screening-level risk assessment that helps identification of knowledge gaps and key drivers of uncertainty, and that will support hypothesis generation to prioritize future environmental monitoring and process studies.

### **3.02.P-We126 A mass- balance model analysis of Small Microplastics (<100 µm) in highway stormwater runoff**

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Microplastics are ubiquitous in different urban and remote environmental compartments. Among them, the smallest microplastics (SMPs <100 µm) can be more easily transported in the environment, influencing the equilibrium of all the biogeochemical cycles, altering ecosystem dynamics and posing risks for biota and human health. Trafficked highways are considered one of the most important SMP sources since the highway stormwater runoff can wash off these pollutants emitted from vehicles, atmospheric deposition and other sources and reach receiving water bodies. However, there is a general lack of scientific literature about their analysis and consequently to their source, transport and fate pathways processes in the environment. Hence, a modelling analysis can overcome this lack of the SMP measurements and the discrepancies in literature data, due to different methods employed, units of measure and errors). These assumptions can be critical, as pollution levels in stormwater discharges are known to have a strong inter-event variability for micropollutants (and supposed to be also for SMPs). In this work, we developed a first dynamic event-based modelling approach to estimate the SMP fluxes from highway stormwater runoff, identify the most relevant pathways and show how the model can be used to assess the impact of end-of-pipe stormwater control measures on SMPs' emissions to the receiving water bodies. In this model, we employed a detailed novel SMP database based on our previously published studies, where we provide the temporal behaviour of the different SMP sources (road dust, dry and wet deposition) in the highway system context. The model results are extremely important to support the development of future pollution control options and strategies in the urban context, for instance for identifying and implementing the most cost-effective measures to decrease SMP emissions.

### 3.02.P-We127 Fugacity-based estimation of contaminated areas in coastal wetland receiving oxytetracycline from livestock wastewater

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Oxytetracycline (OTC) is an important antibiotic used in the treatment and prevention of diseases in animal husbandry. Improper use and overuse of antibiotics leading to antibiotic resistance problems in the ecological environment and human health is an unignored problem. In Taiwan, current laws and regulations have not established standards for the discharge of antibiotics in livestock wastewater. Moreover, it is difficult to understand the potential impact of the use of OTC in the livestock industry on the water environment due to a lack of information on its environmental fate. Therefore, the aim of this study is to estimate the area of coastal wetlands affected by OTC discharged from drainage by quantifying OTC concentrations and fugacity model to provide implications for antibiotic management in coastal aquaculture. We collect water samples from drainage and coastal wetland and quantify OTC concentrations. On the other hand, a fugacity model is constructed to estimate the emission rate of OTC and to solve the area of contaminated coastal wetland based on the actual measurements. Results show that the average measured OTC concentration is  $1.62 \times 10^{-6} \text{ mol m}^{-3}$ . Based on the developed fugacity model, emission rate is estimated as  $2.68 \times 10^{-1} \pm 3.31 \times 10^{-1} \text{ mol h}^{-1}$  and the area of contaminated coastal wetland is estimated as  $8.19 \times 10^{13}$  or  $6.69 \times 10^3 \text{ m}^2$ . It should be noted that the area of the study coastal wetland is  $\sim 3.8 \times 10^7 \text{ m}^2$ . Here we apply the actual OTC concentrations in drainage and coastal area to estimate the range of contaminated coastal areas by the developed fugacity model. Although it is needed to further discuss the true contaminated range, the study framework could be further used in coastal aquaculture management and provide implications for environmental policies.

### 3.02.P-We128 Improving the Parameterization of Forest Filter Effect in Environmental Fate Models: A Meta-Analysis Review of Particulate Matter/Leaf Interaction

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Particulate matter (PM) consists of different inorganic and organic components, and their aerodynamic diameters are in the range of 0.001–100  $\mu\text{m}$ . PM is often associated with chemicals such as polycyclic aromatic hydrocarbons (PAHs) which can adsorb on it, especially the high molecular weight ones. PAHs originate from natural and anthropogenic sources and are among the most common air pollutants, especially in cities and industrial areas. For this reason, the improvement of the parametrization of the PM/leaf interaction is crucial for better understanding the role of plant biomass in the removal of PM (and PAHs) from air. While the main processes are currently accounted for in the available fate models (accumulation, dry and wet deposition of particles, rain dissolution, particle and wax erosion, degradation of chemical and mineralization of organic carbon, etc.) much is still to be done to fully understand the mass balance of PM in different environmental conditions (PM size classes and concentrations, meteorological parameter, species specific parameters such as leaf area index, specific leaf area, etc.) In this review, we collected and organized the available information on PM interaction between air and leaves. The analysis was performed on papers published between 1970 and 2023. For example, deposition velocity ( $V_d$ ) data were collected, and this showed that many parameters are important in determining the extent of deposition velocity values: wind speed, differences in PM sizes. Among the environmental, scenario driven parameters of different ecosystems, some factors including the vicinity of plants to contamination sources, canopy morphology, period of exposure to pollution, meteorological conditions, orientation, surface area, cuticle roughness and integrity, and the presence of hairs on the surface of the leaves could influence the absorption and accumulation of particulates by leaves. This review will show and compare the available data influencing air/leaf PM accumulation as well as their association with PAHs. The objective is then to provide guidance to properly select simulation parameters for calculating PM mass balance in urban forests and choose the best species to mitigate PM pollution, especially in cities.

### 3.02.P-We129 Mass Balance Equations Model To Assess Environmental Fate of Micro- and Nanoplastics

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The presence of enduring plastic waste is ubiquitous, resulting in its widespread accumulation in the environment. Over time, this waste undergoes gradual degradation into micro- and nanoplastic particles (MNPs), characterized by dimensions ranging from 0,1  $\mu\text{m}$  to 5 mm (microplastics) and less than 100 nm (nanoplastics). MNPs constitute an intricate amalgamation of polymers and chemicals intentionally incorporated into plastics to enhance their performance, functionality, and aging properties. Owing to their hydrophobic nature and small size, MNPs may also serve as vectors for the transmission of other hazardous substances into the human body (heavy metal, emergent contaminants as antibiotics and drugs, or pathogens such as bacteria).

Despite substantial advancements in the analysis of microplastics in environmental contexts, nanoplastic particles have rarely been addressed so far. Indeed, the examination of nanoplastics in intricate samples remains a formidable challenge for existing analytical techniques, necessitating significant enhancements in sample preparation methodologies. Consequently, a modelling approach that prognosticates the environmental fate of MNPs emerges as a crucial tool for comprehensive safety assessments.

In this context, we introduce SimpleBox4MNPs, an environmental fate model conceived as part of the PlasticsFatE project. This model is designed to predict the environmental destiny of MNPs, adapting the SimpleBox4Nano model to accommodate the specific behaviours of microplastics (MP) and nanoplastics (NP). Analogous to its predecessor, the new model is based in

mass balance equations and primarily seeks to elucidate the fate of MNPs released into the environment, encompassing emissions into atmospheric, surface water, soil, and sediment compartments.

Significant improvements include an approach to the structural layer force and the consideration of particles larger than 100 nm, facilitating the differentiation of aggregation mechanisms between microplastics and nanoplastics. Additionally, the model incorporates a cascading fragmentation model to simulate size distribution, enabling the prediction of nanoplastic formation from microplastics.

In conclusion, the utilization of computational tools represents a distinct advantage in the realm of risk assessment, facilitating a comprehensive understanding of the ultimate fate and, subsequently, the anticipated exposure to MNPs in diverse environmental settings.

### **3.02.P-We130 Developing a Modelling Approach to Estimate Exposure of Coastal Birds to Heavy Metals: A Case Study of the European Shag *Gulosus aristotelis***

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Many ecosystems are exposed to heavy metals due to emissions from a range of sources including industry, historic wastes, transportation or agricultural practices. Much modelling work has been done to assess how metals bioaccumulate in various species and food webs. However, very little work has been done to incorporate bird species into these models. At present, most avian risk assessment studies rely on empirical concentration data being collected directly from birds, which requires invasive sampling. Here, we present an approach to predict the exposure of a coastal bird species (European shag *Gulosus aristotelis*) to six metals (Cd, Cu, Pb, As, Ni, Zn), using an existing bioaccumulation model (MERLIN Expo) in conjunction with a newly developed avian model. Seasonal metal concentration data for sediment and plankton were obtained for two sites in the Firth of Forth estuary (Scotland), which represent a hypothesised exposure gradient and host two distinct populations of the European shag at the Isle of May (lower estuary) and Inchkeith (upper estuary). These data were used as model inputs to predict the bioaccumulation of each metal in 14 aquatic prey items at either site, for both seasons. Estimated prey concentrations were then used with avian site-specific biometric data and season-specific dietary data to estimate metal exposure (mg/day or mg/kg bw<sup>-1</sup>) in individual shags belonging to either population, for both the summer and winter. Finally, the estimated daily exposure was compared to available avian toxicity thresholds (Zn, Cu and Pb only) to establish the level of risk. Patterns of bioaccumulation in prey species differed between the two sites; Ni presented higher rates of bioaccumulation in benthic species. Daily exposure for individual shags was predicted to be higher at the Isle of May compared to Inchkeith for all six metals, with Cu showing the largest difference between the two. Exposure was also found to be greater in winter compared to summer. In terms of risk, avian toxicity thresholds were surpassed for Zn and Cu in both populations, in both seasons. Further work is planned to evaluate this model using existing avian samples collected as part of a long-term monitoring programme for European shags in the Firth of Forth estuary. This approach could be useful as a risk assessment tool and/or to develop more sensitive predictions of metal exposure in coastal/marine bird species where population-specific data are available.

### **3.02.P-We131 Mechanistically Modeling the Long-term Human Exposure to Chlorinated Paraffins**

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The extensive production and widespread application of chlorinated paraffins (CPs), including short-, medium- and long-chain CPs, have led to their ubiquitous occurrence in the environment and subsequent human exposure. However, a critical knowledge gap persists regarding how the application patterns and temporal trends in environmental release can shape the long-term human exposure to CPs. Here we are using a modelling approach to examine and contrast time-variant human exposure of the general populations in three regions with differing application patterns and release trends for CPs — western Europe, China, and North America. We hypothesized that in regions where CPs are predominantly applied outdoors, such as in metalworking fluids (e.g., western Europe), environmental release occurs primarily outdoors, making far-field exposure more relevant. Conversely, in regions where CPs are primarily used indoors, such as in polyvinyl chloride products (e.g., China), dominant indoor release emphasizes the importance of near-field exposure. By using the model to link environmental release with human exposure, we examine: (i) how the temporal trends of human-body burdens in longitudinal cohorts vary among these regions, and how they depend on CP environmental release; (ii) whether the relative importance of far-field vs. near-field exposure differs due to varying application patterns; and further (iii) how any such difference in relative importance changes among different age cohorts and with various CP homologue groups. This work offers a pioneering and comprehensive understanding of long-term human exposure to CPs and identifies key factors driving this process. Furthermore, the integration of exposure modeling with emission estimation establishes a scientific foundation for identifying key exposure sources, efficiently managing health risks associated with CPs, and assessing the potential effects of future emission reduction strategies.

### **3.02.P-We132 Advancing Dermal Exposure Modeling: Integration, Evaluation, and Comparative Analysis within the EAS-E Suite Framework**

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Quantifying chemical exposure by the dermal route is an important element of safety evaluations for product stewardship and regulatory decision-making. Recent risk evaluations under the Toxic Substances Control Act (TSCA) have highlighted the need for improving the scientific basis of dermal exposure modelling used in evaluating the dermal route of exposure for workers and consumers.

The objective of this presentation is to address uncertainty in the selection of appropriate dermal exposure models for decision-making. We have integrated a suite of dermal exposure models within the Exposure And Safety Estimation (EAS-E) Suite framework, facilitating the comparison and evaluation through a series of case studies. Chemical properties and parameters required to run the models are directly provided within the EAS-E Suite platform to facilitate model parameterization. The dermal exposure models included in EAS-E Suite are those used in the RAIDAR-ICE and PROTEX-HT models, American Industrial Hygiene Association's SkinPerm, US EPA's Consumer Exposure Model (CEM), as well as ECETOC's Targeted Risk Assessment (TRA) tools for Consumer and Worker Exposure.

Dermal exposure assessment can be conducted (i) as a "stand-alone" analysis, where models exclusively simulate chemical exposure through direct skin contact in specific scenarios, or (ii) as part of comprehensive multi-pathway exposure models, where dermal exposure is integrated into an aggregate exposure assessment. Some dermal exposure models focus on estimating the dermal absorbed dose within defined exposure scenarios while others are coupled with toxicokinetic models, enabling the calculation of systemic exposure metrics such as blood and urine concentrations following dermal or aggregate exposures.

The analysis of the different models has revealed that the primary equations for the dermal permeation coefficient ( $k_p$ , cm/hr) in most of the cases is the "SKINPERM" model developed by ten Berge. This agrees with previous analysis indicating that the "SKINPERM" Quantitative Structure-Activity Relationship model is the best performing model available for estimating  $k_p$ . Furthermore, the "SKINPERM" model can be parameterized using only the octanol-water partition coefficient ( $K_{ow}$ ) and molar mass facilitating the application for thousands of data poor chemicals. Different data sets capturing a range of chemical properties and exposure scenarios (occupational vs consumer) were used for model comparisons and evaluations.

### **3.02.P-We133 Risk Assessment of UV Filters from Sunscreens and Other Cosmetic Products in Recreational Waters: A Case Study of Southern Poland**

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UV filters are commonly used in Personal Care Products (PCPs) as they are essential for skin protection from sunlight. Despite being still poorly researched, due to their wide application hundreds of tons of UV filters are released into the environment each year, thus they are considered recently as emerging contaminants. Lately concerns were raised on their distribution and bioaccumulation in the various trophic levels, and even in humans as some UV filters act as Endocrine Disrupting Chemicals (EDCs).

This research aimed to provide screening studies on the UV filters in popular recreational water reservoirs in Southern Poland, and in the Dobczyce Reservoir as the reference site. The sampling campaigns were carried out in both winter and summer months reflecting lack and peak recreational activity, respectively. In the selected sampling points of each reservoir, water, suspended matter, and bottom sediment samples were collected.

The contents of the most often used in PCPs UV filters were investigated in our study, both organic: benzophenone-3 (BP3), 4-methylbenzylidene-camphor (4-MBC), octocrylene (OC), ethylhexyl methoxycinnamate (EHMC), butylmethoxydibenzoylmethane (BM-DBM), homosalate (HMS), methylene bis-benzotriazolyl tetramethyl butylphenol (MBBT), isoamyl methoxycinnamate (IAMC) and inorganic: titanium dioxide ( $TiO_2$ ) and zinc oxide (ZnO). Analyses of UV filters were performed using Gas Chromatography Mass Spectrometry (GC-MS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), X-Ray Fluorescence (XRF), and Scanning Electron Microscopy (SEM).

Based on the UV filters contents the environmental and health risk assessment will be performed, using the US Environmental Protection Agency (USEPA) methodology and employing Monte Carlo modelling (DecisionTools Suite Lumivero) in order to determine variability and uncertainty of the results during current exposure.

The results of these preliminary studies in Southern Poland will bring new insights into temporal and spatial UV filter content changes in the recreational reservoirs, in both potentially polluted and unpolluted environments. As the threshold levels of UV filters are missing in the regulatory acts, the results of our studies might be meaningful in establishing the acceptable risk levels for this group of emerging contaminants.

### **3.02.P-We134 Environmental Risk Assessment Screening Proposal – applying country-specific dilution factors and refined input factors**

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Exposure assessment approaches for Human Safety Assessment differ significantly from respective Environmental Safety Assessments. Most Human Safety Assessments are concentration-based and focus on setting safe limits for individual products to protect consumers from potential health risks. The standard approach for Environmental Risk Approaches takes the total tonnage of a chemical (or the group of chemicals with the same mode of action) into account to evaluate and manage the environmental impact across all industries and applications. In cases where chemicals have different applications and uses with different exposure scenarios, we advocate for a combination of the two complementing approaches to facilitate environment-responsible ingredient decisions. Such a concentration-based approach can be applied and validated with conclusions from data-rich substances, e.g. alcohol-ethoxylates.

The HERA (Human & Environmental Risk Assessment on ingredients of European household cleaning products) report for Alcohol Ethoxylates (version 2.0, September 2009) includes an extensive Environmental Risk Assessment (ERA) for these chemicals. We developed an ERA screening for data poor chemicals and used the HERA data as a safety benchmark for our evaluation of Alkyl-PEGs. Predicted Environmental Concentrations (PECs) are derived for surface water and soil. Respective Predicted No-Effect Concentrations (PNECs) were derived for freshwater and soil applying standard methods and defaults.

In a first step towards refining the Environmental Risk Assessment we applied country-specific wastewater volumes and dilution factors to overcome the limitations of the overall default value. In a subsequent step we refined the input factors taking demographics and use-patterns into account.

By applying this proposed ERA screening method, we are confident that we can effectively identify critical ingredients within a larger portfolio of chemicals, leading to a more refined and accurate assessment of their potential environmental risks and ultimately supporting the development of more sustainable and environmentally friendly cosmetic formulations.

### **3.02.P-We135 Considerations for Applying the Parallel Artificial Membrane Permeability Assay (PAMPA) in the Screening of Gastrointestinal Absorption of Chemicals of Environmental or Occupational Concerns**

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Chemicals present in the environmental and occupational settings can exert adverse health effects on humans after gastrointestinal absorption into the systemic circulation. Parallel Artificial Membrane Permeability Assay (PAMPA) has gained advocacy for its application to measure the effective permeability towards pharmaceuticals. However, since chemicals of environmental or occupational concerns differ from pharmaceuticals in hydrophobicity and volatility, a thorough, mechanistic understanding of chemical mass transfer in PAMPA is warranted if we seek to expand the applicability of PAMPA. Here, we introduce an in-silico mass balance model, which describes chemical mass transfer in PAMPA based on inputs of fundamental physicochemical properties, e.g., molecular weight, partition coefficient, and dissociation constant. The model's performance is evaluated by an agreement between predicted and measured permeabilities of 1383 chemicals, which indicates that 95% of the estimated permeabilities are either fall in the same order of magnitude or more conservative than the measurements. The model predicts an inverted U-shaped dependence of permeability on the octanol-water partition coefficient ( $\log K_{OW}$  for neutral compounds and  $\log D_{OW}$  for ionizable compounds), with the maximum permeability occurring in  $\log K_{OW}$  or  $\log D_{OW}$  ranging between 0 and 2. The model estimates a high membrane retention rate for hydrophobic chemicals, as well as the loss of volatilization to the headspace of the PAMPA apparatus for highly volatile chemicals. Notably, the measured permeabilities of hydrophobic chemicals are remarkably sensitive to specific experimental conditions, e.g., frequency of stirring, and incubation time, making measurements under different conditions less comparable. More important, for highly hydrophobic chemicals ( $\log K_{OW}$  or  $\log D_{OW}$  greater than 3.8), steady-states mass transfer, which is the fundamental assumption of PAMPA can never be achieved. Therefore, the current design of PAMPA needs to be modified before its application to chemicals of environmental or occupational concerns. Our work provides an in-silico mechanistic approach in support of efficiently and defensibly predicting the permeability of chemicals and complements the current laboratory approach.

### **3.02.P-We136 An Extension to Current Model Averaging Methods for Benchmark Dose Estimation**

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In dose-response modeling, several models can often yield satisfactory fits to the observed data. In this case, the current practice used in risk assessment is to combine the fitted models using model averaging, which is a way to combine several models in a weighted average. One particular important parameter, used in risk assessment, is the benchmark dose, which is the dose level resulting in a predefined abnormal change in the response.

Current practice when applying model averaging is to use weights based on the Akaike Information Criterion of the individual models. We introduce an alternative to these weights, denoted the Stacking weights, which is the set of weights minimising the squared prediction error of the model averaged curve.

A simulation study was conducted to assess the performance of the Stacking weights compared to the currently used weights. The simulation study showed that the currently recommended weights performed well. The newly introduced Stacking weights performed similarly. Based on the promising results of the Stacking weights, they have been added as a feature in the R package "bmd".

### **3.02.P-We137 Daphnids response to physical toxicants; individual response and data capture approaches**

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Physical toxicants in the environment, such as nanomaterials and microplastics, can pose an interesting challenge to organism once ingested. The observed toxicity can be due to a combination of factors- the physical presence of the toxicant and any associated inflammation response, the calorific deficit that is induced through the ingestion of particles reducing the food uptake, and the additional energy requirement to remove and depurate the particle where possible. Through this combination, there could be various changes to the available energy, which will be interesting to explore through dynamic energy budget models, taking into consideration toxicity responses and also starvation effects.

Daphnia are an excellent model through which to explore this, as they are filter feeders and therefore a realistic model organism to explore freshwater particle toxicology. Daphnia have also widely been used in ecotoxicity studies previously, for a range of toxicants including nanomaterials and microplastics, in addition to other environmental parameters such as food shortages. Life history data of Daphnia has also widely been explored and there is a wealth of data available as a starting point to develop the model.

This project explores that use of Daphnia chronic toxicity data (OECD 211) from a microplastics and surfactant co-exposure test in a Dynamic Energy Budget model, and explores the data capture templates and end points that would be useful to strengthen the experimental design, and model in due course. For example, ensuring that the individual Daphnid size is recorded at the release of the first brood is a useful measurement for the parameter estimation for the model, and including this in a data capture template for chronic exposures going forwards would be a useful, and not excessive, addition to chronic Daphnia exposure observations. By generating and creating comprehensive data capture templates, we can support the FAIR data principles, by facilitating the use of ecotoxicological exposures into more complex environmental questions going forwards.

### **3.02.P-We138 Environmental risk assessment of different forms of graphene-based materials in European freshwaters**

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The increasing use of graphene-based materials (GBMs) in diverse applications has been raising environmental concerns. Our previous study performed a material flow analysis, identifying GBM release pathways and estimated concentrations in various environmental compartments. Although the importance of considering forms of GBMs in realistic characterization of the environmental risk assessments of pollutants, the different forms of GBM, including pristine graphene, graphene oxide, and reduced graphene oxide, has not been accounted in the environmental risk assessments yet.

To address this critical gap, we propose a form-specific approach for environmental risk assessment. This approach combines predicted environmental concentration (PEC) derived from MFA with a predicted no-effect concentration (PNEC) calculated from the form-specific Probabilistic Species Sensitivity Distribution analysis to derive the risk characterization ratio (RCR). By calculating form-specific risk characterization ratios of individual GBMs, our study aims more accurate environmental risk assessment and enables to inform effective risk management strategies.

Our study reveals that the PECs of all forms of GBMs are extremely low. The PNECs of different forms of GBMs are comparable, indicating similar toxicity of different forms. Furthermore, the RCRs for all forms of GBMs are well below 1, indicating a negligible environmental risk. This study significantly enhances our understanding of the potential environmental risks associated with GBMs and lays the groundwork for further investigations into their actual environmental impact. By providing a form-specific approach for assessing the environmental impacts of GBMs, our research contributes to a more sustainable future by reducing uncertainties, improving decision-making processes, and enhancing environmental protection.

### **3.03.A Advances in High Resolution Mass Spectrometry Based Non-targeted Analysis for Exposure Monitoring and Assessment of Human and Environmental Samples**

#### **3.03.A.T-01 Automated Prediction of Toxic Chemicals in Complex Mixtures**

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Exposure to toxic chemicals, including endocrine disruptors, poses significant risks to both ecological and human health. To discover such chemicals, high-resolution mass spectrometry (HRMS) is commonly employed. Simultaneously, samples are complicated chemical mixtures where thousands of HRMS features are detected. Pinpointing and identifying the chemicals causing the toxic effects remains time-consuming, and often only a fraction of the toxicity is explained.

To address this, we are developing *in-silico* data analysis tools for risk-based prioritization using HRMS features of unknown chemicals. We hypothesize that combining unsupervised and supervised learning allows extracting toxicity-relevant information from the tandem mass spectra.

Unsupervised molecular network approaches, based on HRMS spectra from compounds with known toxicity, offer a promising strategy for extracting structure and toxicity-relevant information independent of direct library matching. Each HRMS spectrum represents a node; similar spectra are linked with the edges. Detected HRMS spectra from samples can be mapped to the developed network, allowing a spectral similarity-based toxicity evaluation prior to the identification of the detected features. The network approach yields sensitivities above 0.6 for nr.ahr and nr.er.lbd targeting assays.

Furthermore, we evaluated the combination of deep-learning autoencoders coupled with random forest-based toxicity prediction. We found that autoencoder models are capable of extracting compound-specific information with the ability to reconstruct the original spectrum from the compressed latent space information with an average cosine similarity of 0.73. Notably, these models exhibit a reduced emphasis on low-intensity peaks, hinting at their potential application in spectral noise removal. Ongoing research aims to enhance these approaches, providing numerical results by the time of the conference.

### **3.03.A.T-02 A stochastic approach for parameter optimization of feature detection algorithms**

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#### **Abstract:**

Feature detection plays a crucial role in non-target screening (NTS), requiring careful selection of algorithm parameters to minimize false positive (FP) features. In this study, a stochastic approach was designed and evaluated to optimize the parameter settings of feature detection algorithms used in processing high resolution mass spectrometry data for NTS. This approach was validated by its application to four open-source algorithms (OpenMS, SAFD, XCMS and KPIC2) within patRoon platform for processing extracts from spiked drinking water samples containing 46 per- and poly-fluoroalkyl substances (PFAS). The designed method is based on stochastic strategy involving random sampling from variable space and the use of Pearson correlation to assess the impact of each parameter on the number of detected suspect analytes.

Using our optimizing approach, the optimized parameters led to improvement in the algorithm performance either by increasing suspect hits in case of SAFD and XCMS, or reducing the total number of detected features (i.e., minimizing FP) for OpenMS. These improvements were further validated on three different drinking water samples as test dataset. The optimized parameters resulted in lower false discovery rate (FDR%) compared to default parameters. This approach effectively increased the detection of true positive features (confirmed across different scenario of defining the true positive), and underscored the advantage of using the consensus of data from multiple algorithms to prioritize the overlapped features as a true feature to be subsequently used in the NTS. This work also indicated the necessity to perform parameter optimization initially in the NTS workflow, using a sample spiked with the targeted group of chemicals to optimize algorithm parameter settings, by maximize the detection of the true-positive features (suspect hits).

### **3.03.A.T-03 A data analysis pipeline integrating ion mobility and high-resolution mass spectrometry for non-target screening in environmental studies**

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The goal of this study is to develop a pipeline for analysis of HRMS data including ion mobility in the field of environmental studies, to overcome the challenges posed by proprietary vendor software and to take advantage of recent open data formats and softwares. Especially, it was developed to avoid alignment problems and data format limitations encountered with proprietary software. The pipeline includes a condensed and convenient data format (Apache Parquet) to allow for the compatibility of data with various programming languages and open-source packages. For example, data can be imported directly in DEIMoS, a recent Python package allowing the processing of HRMS data with ion mobility. It incorporates fast signal processing algorithms for peak detection and alignment. The processed data will be further used with other open-source packages used in environmental studies, such as patRoon, to enable extensive interrogation of a number of spectral libraries for the identification of unknown compounds. This comprehensive pipeline will be implemented in HRMS analysis of environmental samples, especially for surface waters impacted by urban wet weather discharges of wastewater. It is anticipated to facilitate the identification of contaminants of interest in polluted waters, as part of environmental monitoring performed in the Paris region. The pipeline will also be used in conjunction with ecotoxicological analyses for extensive data modeling of toxic contamination in complex environmental mixtures.

### **3.03.A.T-04 Exposomics meet Quantitative Non-Target Screening: A tool for Semiquantitative Analysis of Emerging Contaminants in Human biofluids and Tissues**

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The term exposome was firstly coined by C. Wild as life-course environmental exposures from prenatal period onwards, including exposure to exogenous chemicals. However, to holistically understand human external chemical exposome, nontarget strategies are highly encouraged. Nontarget analyses (NTS), despite their unbiased results, have been weighed down in exposomics field by their lower sensitivity compared to low resolution, as well as the lack of other analytical validation characteristics (recovery values, matrix effects (ME) or specificity). However, the main challenge is its qualitative nature. Although classical target strategies are constrained to a small set of pre-selected chemicals, the derivation of accurate concentrations made target strategies the ultimate choice.

Recently, new strategies have emerged to NTS a semiquantitative analysis using machine learning algorithms to learn from how chemicals ionize in the ionization source. Generally, the concept of ionization mode of action is established based on working solvents and it can provide acceptable interpretations on ionization-concentration relationship. Despite recovery values effect can be reduced by using simple sample treatments, the ME is still having an important impact. Therefore, it is necessary for development of specific ionization-efficiency algorithms to compensate for matrix effect in case solvent-based ionization efficiency fails to resolves the bias from ME.

In this work, we have trained six different tools (solvent, human serum, urine, brain, placenta and semen) to obtain semiquantitative results from NTS. Different sample preparation methods have been used: deproteinization strategy with acetonitrile (semen and serum), CAPTIVA cartridges clean-up (urine) or lysis and pre-concentration with SPE cartridges (tissues). Recovery values were satisfactory for almost all tested chemicals, so the method was built to counteract matrix effects in the quantitation process.

Molecular descriptors were filtered using ant-colony optimization, and the best ones were kept training each model. We have tested the tool then with different real non-spiked samples, comparing quantitative (using a real calibration curve) with the semi-quantitative ones, with values between 50-200 % of the accurately calculated value.

Our tool opens the possibility to perform comprehensive NTS of human samples in a semiquantitative manner, virtually obtaining a wide characterisation of the human chemical exposome.

### **3.03.A.T-05 Comprehensive suspect screening workflow for the analysis of xenobiotics and metabolites in human biofluids**

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Suspect and non-target substance screening (SNTS) methods are gaining prominence for deciphering the human exposome due to their ability to analyse a broad chemical spectrum in human biofluids. Nevertheless, SNTS methods using human biofluids are infra studied in comparison to other samples, such as environmental matrices. Therefore, the need of comprehensive workflows with robust and reliable quality control/quality assurance (QC/QA) measures are demanding. In this study, we developed a suspect screening workflow to identify exposome-related xenobiotics and phase II metabolites in diverse human biofluids, specifically, human urine, breast milk, saliva, and ovarian follicular fluid. These samples were extracted with appropriate sample preparation protocols and analysed using ultra-high-performance liquid chromatography coupled with high-performance tandem mass spectrometry (UHPLC-HRMS/MS). The parameters and criteria introduced in the workflow were optimize to ensure the simultaneous annotation of 183 xenobiotics. In that sense, the "peak rating" parameter was compulsory to eliminate the labour-intensive manual review of chromatographic peaks and also to avoid the chromatographic peak area threshold that could eliminate low abundance peaks. Additionally, the impact of endogenous molecules on xenobiotic annotation we carefully examined employing inclusion and exclusion suspect lists in the post-processing step. To assess the workflow's performance, limits of identification (LOI) were determined, as well as type I (false positives) and II errors (false negatives) in both standard solutions and spiked biofluids. Remarkably, 80.3% of the suspects achieved LOIs below 15 ng/mL. Regarding type I errors, only two cases were identified in standards and spiked samples. Conversely, type II errors increased from 7.7% in standards to 17.4% in real samples. Notably, the use of an inclusion list for endogenous molecules proved advantageous, preventing 18.7% of type I errors, while the exclusion list led to 7.2% of type II errors despite the annotation workflow being less time-consuming.

### **3.03.B Advances in High Resolution Mass Spectrometry Based Non-targeted Analysis for Exposure Monitoring and Assessment of Human and Environmental Samples**

#### **3.03.B.T-01 Harnessing Molecular Ions by GC-APCI-IM-HRMS for Simultaneous Target, Suspect, and Nontarget Screening of Hydrophobic Contaminants in Sediments**

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The increasing diversity of chemicals used by society requires new analytical methods to determine regulated contaminants, while simultaneously collecting nontarget data to identify unknown compounds. However, due to the extensive fragmentation during electron impact ionization (EI), gas chromatography (GC)-based screening suffers from the complexity of fragment ions and infeasibility for *de novo* identification, resulting in the scant census of hydrophobic organic contaminants (HOCs). Herein, atmospheric pressure chemical ionization (APCI) was hyphenated to GC-high resolution mass spectrometry (HRMS) to preserve (quasi-)molecular ions, which are subsequently separated by ion mobility (IM) to derive instrument-independent collision cross section (CCS) for forensic identification. A novel analytical workflow was developed for simultaneous target, suspect, and non-target screening of HOCs using background sediment samples from the Baltic Sea. The chemical space of this method was defined by 87 contaminants analyzed as target compounds with  $1.44 < \log K_{OW} < 16.8$  and  $4.76 < \log K_{OA} < 16.9$ . After optimization of instrumental parameters, 78% of our targets yielded (quasi-)molecular ions as base peaks; instrumental detection limits of GC-APCI-IM-HRMS in MS<sup>E</sup> mode for 86% of our target compounds are similar to or lower than those of GC-MS using EI in selective ion monitoring mode. Four-dimensional information (i.e., formula, CCS, retention time, and fragments) of 463 GC-amenable compounds were collected for suspect screening. Observed CCS of 26 target compounds matched values in libraries, while CCS of 61 target compounds were reported for the first time. From the sediment samples, 12 compounds were annotated through 4D suspect screening using strict criteria. Leveraging CCS as a dimension of size, 705 unknown features were prioritized as potential organohalogenes. This strategy was validated by the identification of natural brominated compounds. The utility of the method was further demonstrated through the application to archived sediment samples with different contamination levels and geographical locations from Arctic shelf seas, the Japanese Sea, and a Norwegian lake. Therefore, GC-APCI-IM-HRMS is a promising next generation technique for resolving complex mixtures of HOCs through fully exploitation of molecular ions. This study also accentuates the urgent need to extend CCS libraries and retrain *in silico* tools for HOCs.

### 3.03.B.T-02 Improved target, suspect- and non-target analysis of environmental contaminants in wastewater using hydrophilic-lipophilic balanced SPME and GC-EI&CI-TOFMS

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Wastewater plants can release aqueous effluents containing harmful pollutants, posing risks to ecosystems and human health. Given the dynamic nature of wastewater composition, regular target and non-target analyses are frequently conducted. Due to its complementary polarity range, gas chromatography mass spectrometry (GC-MS) is often employed alongside liquid chromatography (LC) MS to evaluate potential hazard profiles, to identify compounds and to gain a more complete overview of the potential hazards present. However, conventional GC-EI-MS is limited in its identification confidence for some compounds due to unspecific fragmentation pattern, absent molecular ion signals or by not being available in reference libraries, leading to a lack of compound identification. Additionally, with little to no prior knowledge of sample constituents, a broad extraction method is paramount.

For this study, 24h effluent samples were collected from an industrial plant over two months. Analytes were enriched by headspace solid phase microextraction (SPME) using a novel hydrophilic-lipophilic balanced (HLB) fiber coating (CTC, CH). An 7890A GC (Agilent, USA) was coupled to a newly developed dual ionization source time-of-flight (TOF) MS (ecTOF, TOFWERK, CH). Here, a standard 70 eV electron ionization (EI) source and a medium pressure chemical ionization (CI) source are operated quasi-simultaneously. Furthermore, the system allows for a simple selection of different CI reagents ions (e.g., [NH<sub>4</sub>]<sup>+</sup>, [N<sub>2</sub>H]<sup>+</sup>, [H<sub>3</sub>O]<sup>+</sup>) between chromatographic runs, which enables the adjustment of reactant selectivity and the degree of fragmentation.

This talk will outline different approaches for compound identification by using the simultaneous acquired EI and CI mass spectra. The additional CI information with its selection of different CI reagents ions is shown to be highly valuable for the identification process. The available GC-EI&CI TOF-MS data is further used to adapt the widely used LC-based workflow to report confidence of identification for compounds found in the wastewater samples. Moreover, the novel HLB-SPME material was tested as a solvent-free headspace extraction of the wastewater constituents and shown to extract a broad range of analytes. In combination with the GC-EI&CI TOF-MS, this methodology can be used to complement commonly employed methods such as LC-ESI-HRMS used in industrial wastewater monitoring.

### 3.03.B.T-03 Combining Advanced Analytical Methodologies to Describe Extractable Organic Fluorine in Human Serum

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Per- and polyfluoroalkyl substances (PFAS) have received international attention due to their persistence, ubiquitous occurrence in wildlife and humans, and potential environmental and health impacts. Since >4700 PFAS exist and <1% of these

chemicals are analyzed in human blood, there are growing concerns that PFAS exposure might be underestimated. This concern is supported by studies where the extractable organic fluorine (EOF) in human blood could not be explained by traditional target PFAS analyses. In particular, a study in pooled serum samples, collected from the Tromsø (Norway) population in 1986, 2007 and 2015, showed that EOF concentrations were only partially explained by target PFAS and oxidizable precursors. In the present study, the same extracts were analyzed by direct infusion Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) and liquid chromatography high resolution-Orbitrap-mass spectrometry (LC-Orbitrap-HRMS). These analyses were used for suspect screening for >5000 PFAS and 342 fluorinated pharmaceuticals and estimate their contribution to EOF in human serum. With FT-ICR-MS 365 suspect PFAS were observed. However, by the LC-Orbitrap-HRMS, only 5 suspect PFAS could be identified. Only 1 PFAS suspect, the perfluoro-4-ethylcyclohexane (PFECHS), was confirmed with standards. Since PFECHS is not oxidizable, it was not surprising that this suspect was detectable before and after the TOP assay. PFECHS contributed to 2-4% of the EOF. However, after PFECHS inclusion, PFAS concentrations did not fully explain the EOF found in serum. Screening of fluorinated pharmaceuticals revealed 9 suspects, but only 3 (teriflunomide, lansoprazole and pantoprazole) were confirmed with standards. Additionally, 5 metabolites of these pharmaceuticals were identified. Overall, fluorinated pharmaceuticals accounted for between 0-56 % of the EOF. The portion of EOF explained by pharmaceuticals in 2007 was significantly higher than in 1986, and in 2015 it was significantly higher than in 2007. The use of suspect screening with FT-ICR-MS and LC-Orbitrap-HRMS in combination with the TOP assay facilitated the description of the EOF in pooled serum. While a large portion of the EOF found in pooled serum from the Tromsø population remains unidentified in 1986, the detection and quantification of fluorinated pharmaceuticals and their metabolites in serum from 2007 and 2015 showed that these compounds can contribute significantly to the EOF.

### **3.03.B.T-04 Wide-Scope and Nontarget Screening of Persistent Anthropogenic Chemicals in Swedish Waters using Feature-Based Molecular Libraries**

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A major challenge in nontarget analysis of environmental waters is to identify anthropogenic chemicals in highly complex matrices with abundant substances of natural origin. Using liquid chromatography-high resolution mass spectrometry, we aimed to identify and structurally characterize persistent chemicals of anthropogenic origin in a nation-wide survey of Swedish groundwaters (n=55), surface waters (n=125) and drinking waters (n=95). In combination with a wide-scope target (n=220) analysis, we used a nontarget workflow and a 'persistence-prioritized' feature-based molecular library from municipal wastewaters (n=34) and urban stormwaters (n=16) to identify persistent and frequently occurring organic contaminants in environmental waters. A library was created for unbiased screening of persistent chemicals by comparison of peak area signals in paired wastewater effluent and influent (n=7): 26 % of detected features in wastewaters were classified as persistent (i.e. n=12,115 features with effluent peak area > 80 % of influent) or potentially persistent (i.e. n=1,077 features with effluent peak area 50-80% of corresponding influent). In groundwater monitoring, as an example, we detected 3,043 of the prioritized molecular features in the environmental samples, and these are currently prioritized for identification or *in-silico* structural annotation. However, among these 3,043 features were melamine, 1,3-diphenylguanidine, 1H-benzotriazole, and 5/4-methyl-1H-benzotriazole (confidence level 1), which are known persistent, mobile and potentially toxic chemicals, demonstrating strong promise for this approach for discovery of persistent chemicals in environmental waters. In wide-scope target screening we also detected many pharmaceuticals, pesticides, and industrial chemicals in municipal source samples (e.g. wastewaters) and environmental samples (e.g. groundwaters). With a large nontarget dataset and a novel approach using feature-based molecular libraries refined on detection frequency and persistence, we show that unbiased screening and molecular characterization is possible in high-throughput to detect and identify anthropogenic micropollutants of concern in environmental waters.

### **3.03.B.T-05 Target, suspect and nontarget screening of contaminants in indoor dust with SUPRAS sample preparation**

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Indoor dust has been recognised as a relevant source of indoor exposure to contaminants. The comprehensive characterization of such a heterogeneous matrix and such a variety of compounds is complex and sample treatment is a critical step. The potential of SUPRAS to efficiently extract a wide polarity range of compounds and to simplify and improve the green properties of sample treatment in this area are discussed. SUPRAS made up of inverse aggregates of hexanol in tetrahydrofuran:water mixtures, which have been previously and successfully applied to the target determination of a variety of organic contaminants in different environmental matrices before, were employed. Analysis was done with liquid chromatography and high resolution mass spectrometry. Twelve samples from public buildings (six educative buildings, two food stores, two nightclubs, one office and a coffee shop) were collected in South Spain. A total of 146 compounds were detected by target (~33%), suspect (~55%) and non-target screening (~12%). Around 86% of all the compounds were identified (or tentatively identified) with levels of confidence equal or higher than 3. Novel designer drugs of abuse, unreported organophosphorus compounds and well-known organic contaminants, such as bisphenols, parabens, phthalates and flame retardants are reported. Differences with previous studies on wide screening of indoor dust reveal the influence of the employed databases for data processing and of the extraction method together with the different contamination profiles given by the sample location.

### 3.03.P Advances in High Resolution Mass Spectrometry Based Non-targeted Analysis for Exposure Monitoring and Assessment of Human and Environmental Samples

#### 3.03.P-We139 Case Study on the Development of a Liquid Chromatography-High Resolution Mass Spectrometry Method for Non-Targeted Detection of $\beta$ -Lactam Containing Antibiotics and their Transformation Products

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Antibiotics are prolifically used to treat infections in both humans and animals; their cumulative toxic effects on aquatic organisms are not well understood and their presence might lead to the development of antibiotic resistant bacteria. They are therefore understandably recognised as chemicals of emerging concern (CECs). The  $\beta$ -lactam functionality is considered responsible for antibiotic/antibacterial activity and therefore detection of  $\beta$ -Lactam containing chemicals and their transformation products are of special interest.

In this work a case study is presented for the determination of  $\beta$ -lactam containing transformation products in an environmental fate biodegradation study on a common antibiotic using liquid chromatography-high resolution mass spectrometry. During this analysis a selection of antibiotics were fragmented to elucidate common fragments that indicate the presence of the  $\beta$ -lactam ring. These fragments were then used to detect  $\beta$ -lactam containing transformation products in study samples. The challenges of using this approach on labile moieties is discussed along with a comparison of the benefits and disadvantages of in-source or all ion fragmentation (AIF) over data dependent scanning.

#### 3.03.P-We140 Structural Investigations of Transformation Products of the Anticoagulant Drug and Rodenticide Warfarin

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Warfarin (Coumadin) is one of the most popular anticoagulant drugs used as a therapeutic in humans to prevent thrombosis, atrial fibrosis, and fibrillation since the 1950s. Because of its ability to hinder blood coagulation by blocking vitamin K-dependent carboxylation of blood clotting precursors, it is also used as a rodenticide worldwide. Until today it has been partially substituted by far more potent anticoagulant rodenticides (ARs), so-called superwarfarins.[1] Numerous studies are confirming secondary and tertiary poisoning with ARs in non-target-animals and wildlife. Up to now, relatively little is known about persistence and toxicity of ARs and naturally, nor technically formed transformation products (TPs) in the environment, food chain, and transformations occurring during wastewater treatment.[2] Herein, we present liquid- and gas chromatographic methods coupled to (high-resolution) mass spectrometry for the analysis of warfarin's transformation products. Methodologies such as UV-irradiation, ozonation, and chlorination were utilised to simulate technical water treatment. Resulting compounds were elucidated and examined by numerous analytical methods. Moreover, the oxidative phase I metabolism was mimicked by an electrochemical flow cell to synthesise and confirm major metabolites.[3-5] The further aim is the toxicological assessment of all substances formed, as well as, the quantification of warfarin and its TPs under environmentally relevant conditions employing the introduced methods.

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#### 3.03.P-We141 Identification of Biomarkers for Citrate Plasticizers through Non-Targeted Analysis and Their Application in Urine Samples.

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Plasticizers are chemicals that make plastic flexible, mainly using a group called phthalates. These substances are known to cause endocrine disruption, leading to regulatory restrictions on their use in many countries. As a result, there is a growing trend towards the use of alternatives, with citrate plasticizers being one of them. However, there is insufficient research on the metabolites of citrate plasticizers upon human exposure. Therefore, this study aimed to identify the metabolites of citrate plasticizers by exposing them to human liver microsomes (HLM) and conducting non-targeted analysis. Furthermore, we intend to identify biomarkers through the analysis of urine samples collected for the measurement of plasticizer exposure levels.

The target substances were ATBC and ATEC. After exposure to HLM, metabolites were explored using LC-Q-TOF-MS. The major metabolites of ATBC were identified as ADBC and DBC, while ATEC metabolites were identified as ADEC and DEC. In this study, quantitative analysis was conducted on 124 samples using the commercially available standard substance, DEC, and the synthetically obtained DBC. As a result, the median value for DBC was 38.45 ng/mL, while DEC was 1.54 ng/mL. Additionally, non-targeted analysis revealed the presence of un-synthesized ADBC and ADEC in some samples. Through this study, metabolites of citrate plasticizers were estimated through non-targeted analysis, and their confirmation was achieved using urine samples. Based on this, non-targeted analysis can be employed to explore metabolites and contribute to epidemiological studies for measuring exposure levels of alternative plasticizers.

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### **3.03.P-We142 Harmonizing Sample Pre-Treatment for Multiclass Analysis of Endocrine Disrupting Compounds and its Metabolites in Human Urine – a Target and Suspect Screening Approach**

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Humans are exposed to environmental contaminants that can harm our health. Urine is a convenient and non-invasive biological sample for assessing this exposure. It can be easily obtained and processed, making it a valuable reference in studying environmental contaminants.

Urine contains different matrix components that can interfere with the quantification process of the analytes of interest. As it is a complex matrix, an appropriate pre-treatment of the sample is of vital importance. In addition, given the small amount of biological sample that is usually available, it may be necessary to use the same sample extract for target (TS), suspect (SS), and non-target (NTS) screening analysis, with the compromises that this entails.

Sample pre-treatment for SS and NTS focuses on removing the matrix while extracting the maximum number of compounds and minimizing the matrix effect. Selective pre-treatment is not possible due to the need to extract analytes with different physico-chemical properties. TS aims to eliminate matrix interferences and develop a selective pre-treatment for the compounds of interest. The objective of this investigation is to optimize a sample pre-treatment suitable for both TS and SS, for a multiclass/multiresidue analysis of endocrine disrupting compounds and their metabolites in human urine. Liquid chromatography - low resolution mass spectrometry is used for TS due to its high sensitivity, while liquid chromatography - high resolution mass spectrometry is used for SS and metabolite detection. Different sample treatment methods, including solid phase extraction, liquid-liquid extraction, and dilute and shoot method, have been tested to achieve the objective.

The best process for the TG study was found to be liquid-liquid extraction employing acetonitrile without using glucuronidase to hydrolyze the metabolites. Recovery (R.) values of 97-115 % and matrix effect (M.E.) values of (-13) - 0 % were obtained for bisphenols and R. values of 80-119 % and M.E. values of (-20) - 10 % were obtained for benzophenones, among other compounds.

The importance of the study lies in the harmonization of sample preparation for both types of analysis with the purpose of saving on costs and experimental time. Moreover, it is intended to search new metabolites in order to unravel the different metabolic pathways of them, in addition to achieving its optimal characterization in human urine samples.

### **3.03.P-We143 A metabolomic approach to the effects of contaminants of concern in fish liver cultures**

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Advances in knowledge regarding adverse outcome pathways in ecotoxicological studies have developed hand-to-hand with the refinement of analytical techniques. Resorting to non-target mass spectrometry combined with metabolomics for toxicological assessments has greatly contributed to understanding organisms' coping mechanisms against stressors while allowing an assessment of the impact of persistent contamination of systems or individual-specific traits – numerous scenarios with biological relevance that have been mostly overlooked. To date, most ecotoxicological studies still lack complexity in design, needed to enhance ecological and environmental relevance. To address the issue, New Approach Methodologies (NAMs) come as methodologically advantageous alternatives, enabling high-throughput toxicology, while attending ethical considerations in line with the 3R's principles. The present study proposes to further support the relevance of applying metabolomics, based on untarget mass spectrometry techniques, to provide a deeper and broader understanding of organisms'

exposure to contaminants, while identifying underlying contamination events. To this end, four adult *Sparus aurata* from a local fish farm were acclimated in controlled conditions and fed daily until satiation, which was stopped 48h before sacrifice for liver removal. After careful removal, livers were cut into cubes ~2x2mm, and cultured in DMEM media. Samples were immediately exposed for 24h to environmental contaminants, after which liver cultures were collected, frozen in liquid N<sub>2</sub>, and stored at -80°C until analysis. Experimental conditions included: i) single exposure to 5 concentrations of polystyrene microbeads (~1µm diameter) and 5 concentrations of zinc; ii) 3 direct mixtures of PS and Zn concentrations used in single exposures. Preliminary results show that: i) fish differ in numerous metabolites - analyses are ongoing to understand which are derived from reactions to the contaminants' exposure; ii) there is an early separation of samples by individual fish, rather than by treatment; iii) numerous compounds from unknown sources (eg. Tonalid, used in perfumes; Levofloxacin, an antibiotic) were detected. The present works intend, finally, to further highlight non-target high-throughput analyses as a powerful tool to provide further knowledge for legislative and regulatory processes, to effectively act towards a lesser impact of human activities, safeguarding ecosystems (and human) health.

### **3.03.P-We144 PFAS residues in fish from England and Catalonia: trend, bioaccumulation and dietary exposure**

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals that persist in the environment, bioaccumulate in tissue and have become ubiquitous contaminants. The original chemicals and their degradation products have a negative impact on human health and are a threat to the environment, therefore many countries worldwide recommend they be monitored at some level. It has been recognized that dietary intake is a significant route of exposure for human populations, especially through consumption of fish.

Despite the existence of over 10,000 PFAS compounds, the limited availability of reference standards poses a challenge, with standards available for less than 2% of these substances. To overcome this limitation, non-targeted approaches using liquid chromatography–high-resolution tandem mass spectrometry (LC-HRMS) are crucial, enhancing the analytical coverage of PFAS in environmental samples and aiding in the identification of new compounds and transformation products.

In this study, LC–HRMS will be used for suspect screening and quantitative target analysis of PFAS in the most frequently consumed fish species in England and Catalonia. Sampling will be conducted between January and March 2024 in the main PFAS hotspots in each region. In England, fish samples will be obtained from fish farms close to Liverpool, Birmingham, and London, while in Catalonia, samples will be collected from rivers in the vicinity of Barcelona.

The research aims to compare the trends of PFAS contamination between the two regions, identify emerging PFAS compounds, and determine compliance with current EU and UK Legislation, focusing on Maximum Residue Limits and Tolerable Daily Intakes established by the European Food Safety Authority (EFSA) and UK REACH in the studied populations. Additionally, the study will investigate determinant factors influencing PFAS concentrations, such as trophic level and fish weight.

This form of comprehensive analysis, utilizing LC-HRMS, provides valuable insights into the current status of PFAS contamination in river fish, investigating both environmental and public health concerns. The evaluation can be used to assess the impact of regulations on PFAS and to assist authorities in their decisions on how best to protect the environment and consumers from exposure to PFAS through food.

### **3.03.P-We145 Analysis of Volatile PFAS and Other Contaminants in Plants, Soil and Water Using High Resolution GC/MS and an Accurate Mass PFAS Library**

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Per- and polyfluoroalkyl substances (PFAS) are emerging contaminants that are found in water, air, soil as well as wildlife and attract growing attention. A subset of PFAS that have been detected in the environment can be volatile or semi-volatile in nature. Therefore, a variety of analytical techniques are necessary for their detection. GC/MS is typically used for detecting volatile and non-polar PFAS compounds. However, one of the challenges in PFAS analysis by GC/MS is a lack of publicly available libraries containing a comprehensive selection of the PFAS EI spectra. Therefore, an accurate mass GC/MS library for PFAS detection was created.

Soil and plant samples were sampled from two fields in California that have historically received biosolids as well as an organic field and extracted with methylene chloride and SPME. The drinking water samples were collected at two different locations in California and represented two different water source categories: a small surface water and a mixed surface and ground water. Water samples were extracted on a multi-mode SPE and solvent exchanged to EtAc.

We have screened the water, soil and plant samples for PFAS using an accurate mass library. A variety of chemicals of industrial origin, drugs, PAH, flame retardants, PCB and pesticide residues have also been identified using the NIST Mass Spectral EI Library and Pesticide PCDL.

### 3.03.P-We146 What's in the Dust? GC×GC-MS Based Non-Target Screening of House Dust

*Andriy Rebryk and Peter Haglund, Department of Chemistry, Umea University, Sweden*

Air pollution is responsible for ca. 8.9 million premature deaths every year. In addition, WHO reported that 99% of all people worldwide breathe polluted air in their ambient environment. Moreover, studies showed that European citizens spend up to 90% of their time indoors. Hence, respective outdoor air quality guidelines and indoor air quality guidelines were introduced. These cover a limited number of pollutants, such as particulate matter, carbon monoxide, benzene, PAHs, and chloroethylenes. However, there are >100,000 registered chemicals in use in Europe alone. Those can pose potential health risks. Therefore, it is important to establish what chemicals of potential concern are emitted and what are their levels in indoor media that can accumulate them and to which residents are constantly exposed, such as house dust. For this, a two-dimensional gas chromatography-mass spectrometry (GC×GC-MS) based non-target screening (NTS) workflow was established. Dust samples were collected in three European cities: Umeå (Sweden), Munich (Germany), and Lviv (Ukraine). Additionally, NORMAN Network collaborative trial sample and NIST Standard Reference Material 2585 house dust were analyzed. Ca. 60 mg of each sample were extracted by ultrasonic extraction in two steps: with dichloromethane (DCM) and then with acetone. To clean-up the samples from matrix elements, such as fatty acids, that interfere with the detection of other compounds, solid-phase extraction (SPE) using aminopropyl cartridges was used. SPE cartridge was conditioned with DCM, sample extract was loaded, and eluted as two fractions: Fr. 1 with DCM and Fr. 2 with methanol:DCM (1:1, v/v). Both fractions were analyzed using LECO Pegasus BT 4D GC×GC-TOFMS. The MS data were matched against NIST23 spectral library, filtered, and aligned. Preliminary results showed >2500 tentatively identified compounds (NIST23-matched features) in the five samples. These were divided into 43 classes. One of the most abundant constituents of Fr. 1 were plastic additives phthalates, such as di(2-ethylhexyl) phthalate, whereas Fr. 2 contained, among other compounds, a mixture of tertiary amines known as DIMLA 1214, whose increased use was reported during the COVID-19 pandemic. Principal component analysis demonstrated close grouping of the Munich, Lviv, and NORMAN samples, whereas the Umeå and NIST samples were well-separated from the aforementioned and from each other, suggesting unique sets of contaminants.

### 3.03.P-We147 Quantitation and screening of drugs in indoor dust from different environments in Spain

*Cristina de Dios Pérez, University of Cordoba, Spain*

#### Quantitation and screening of drugs in indoor dust from different environments in Spain

In this study a novel microextraction approach based on supramolecular solvents (SUPRAS) is employed for sample preparation of indoor dust prior to the determination of drugs by with liquid chromatography and high-resolution mass spectrometry. 45 drugs of abuse and 34 pharmaceuticals were quantitated. SUPRAS of different composition were tested for maximum recoveries. Samples from different environments (offices, classrooms from education buildings, hairdressers, nightclubs, cafes and restaurants) were collected in South Spain and analysed. Acquisition for target and suspect screening was performed with independent data acquisition (IDA) and libraries from the equipment vendor containing retention time values under the same chromatographic conditions. Identification criteria for was determined on the basis of exact mass ( $\leq 5$  ppm) and isotopic pattern fit ( $m\text{Sigma} \leq 50$ ) of both major parents and fragment ions and retention time fit ( $\pm 0.25$  min).

Results showed the ubiquity of drugs of abuse, such as cocaine and cannabis and the identification of novel designer drugs. These results show the ubiquity and high levels of drugs in indoor dust, which have been scarcely reported and it could constitute a relevant source of human exposure.

### 3.03.P-We148 Development of a tandem mass spectral library for source fingerprinting of monoterpene derived organic aerosol in Beijing

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Exposure to fine particulate matter (PM<sub>2.5</sub>) can lead to severe human health effects, with the Global Burden of Disease study estimating PM<sub>2.5</sub> contributed to 4.2 million premature deaths globally in 2015. Organic aerosol (OA), a complex mixture of thousands of compounds, from a variety of sources is the dominant source of PM<sub>2.5</sub> in many regions. Liquid chromatography (LC) coupled to high resolution tandem mass spectrometry (HRMS<sup>2</sup>) is a powerful technique for both targeted and non-targeted analysis of OA. However, due to the complexity of OA samples and lack of authentic standards, precursor source identification, structural elucidation and subsequent quantification is challenging.

This study outlines the development of an open-access tandem mass spectral library known as **Open Access Organic Aerosol (OA<sup>2</sup>)**, for the improved identification of OA species from a range of precursors. Using a flow reactor, monoterpenes underwent photo-oxidation to produce OA, which was collected and analysed using LC-HRMS<sup>2</sup>. By combining MZmine 3 and SIRIUS outputs, a spectral library file containing features with tandem mass spectral data and a predicted molecular formula was generated from each sample.

An untargeted MZmine methodology was then applied to ambient samples collected in summertime Beijing. The untargeted method allowed for the detection of features which were then screened against OA<sup>2</sup> library files. This allowed for the matching of MS<sup>2</sup> data between the ambient sample and species within the library with a known precursor. This methodology increased the confidence of source identification of analytes within the sample and highlighted significant monoterpene derived OA formation in Beijing.

The aim of developing OA<sup>2</sup> as open source is to involve the wider community in developing the next stage of the library, the inclusion of more precursors and mixtures from both biogenic and anthropogenic species, collected across a range of different atmospheric oxidative conditions.

### **3.03.P-We149 An robust protocol for non-target analysis of PFAS in drinking water: from qualitative identification to quantification of unknowns**

*Yong-Lai Feng, Anca Baesu and Yan Li, Health Canada, Canada*

Per- and polyfluoroalkyl substances (PFAS) are a class of thousands of synthetic chemicals, many of which have been used worldwide to make products resistant to water, heat, and stains since the 1950s. Studies show that exposure to even trace amounts of some PFAS is linked to harmful health effects. The EPA has set a health-advisory level of 70 ppt for the combined concentration of PFOA and PFOS in drinking water. The current monitoring program in Canada only targets the most researched PFAS and the number of PFAS characterized in exposure assessments is still very small compared to the total number registered for commercial use, let alone their transformation products and metabolites in the environment. Non-target analysis (NTA) has emerged as a tool used to identify and prioritize chemicals for human exposure assessment. Unlike targeted analysis, there are no established processes for NTA method development and validation, and although efforts have recently been made to harmonize NTA workflows, knowledge gaps still remain. While the sample preparation step determines the chemicals that are extracted, data acquisition and analysis determine chemical identification. Furthermore, quantitation of unknown PFAS without reference standards remains another challenge. Therefore the goals of this study were to develop an robust NTA method from sample preparation to data analysis to provide information on from qualitative identification to quantitative levels of unknown PFAS in drinking water. The influence of data acquisition methods on PFAS identification was evaluated. The study showed that several new PFAS alcohols in drinking water were identified, indicating that PFAS could be transformed to their unknown transformation products in the treatment process. In this study, FluoroMatch was compared with other data screening tools for identification accuracy. A retention time prediction model was used to increase confidence in the identification. A newly developed quantification model was also used to provide quantitative information for those unknown PFAS following the targeted analysis protocol without using their authentic reference standards. The results showed that NTA can be potentially standardized from qualitative identification to quantitation of unknown compounds as in targeted analysis. Therefore, NTA can be a powerful tool in both rapid screening/prioritization and quantitation of unknown and emerging contaminants for human exposure assessment.

### **3.03.P-We150 Highly sensitive quantification and selective identification of contaminants with a TOF high resolution instrument**

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In order to ensure safety in the global water supply, testing for adherence to federal and international requirements is necessary. The tests monitor for chemical residues is necessary. Traditionally, contaminate residue analysis has been performed by triple quadrupole mass spectrometers, due to their selectivity, sensitivity and quantitative power. Accurate mass instruments can afford additional levels of confirmation, however they have traditionally suffered from a lack of sensitivity and precision, especially when performing MS/MS experiments to meet the testing requirements for the regulatory guidelines.

In this poster, both quantitation and screening performance will be demonstrated by using a SCIEX ZenoTOF 7600 system with scheduled Zeno MRM<sup>HR</sup>. The use of the Zeno trap on the ZenoTOF 7600 system allows for >90% of duty cycle related losses to be recovered during MS/MS experiments across the entire mass range. Experimental gains correlated strongly with theoretical gains during this analysis. Enabling the Zeno trap yields a minimum of 5x intensity gain, with gains up to 13x as fragment masses decrease. Further, since the selectivity afforded by accurate mass MS/MS analysis often results in little to no chemical noise, the gains in signal-to-noise approached the gains observed in raw signal.

The developed quantitative method was applied to a variety of environmental samples in order to compare to traditional triple quadrupole mass spectrometry.

Advantage of high-resolution instrument to screen unknown samples and identify unknown substances by using different workflows will be also demonstrated here.

### **3.03.P-We151 Study of Tap Water Potential as a New Source of Contaminants of Emerging Concern for Population Exposure**

*Luis Muñiz de Bustamante, University of Cordoba, Spain*

The continuous overproduction of chemicals leads to the release in the environment of thousands of chemical compounds. Many studies have found evidence that wastewater effluents contain tens of thousands of organic pollutants, highlighting the impossibility of eliminating all contaminants in wastewater treatment plants. For most of these compounds, chemical and toxicological information is either scarce or unknown, so they are known as chemicals of emerging concern (CEC). These

effluents are discharged directly into different water bodies, where the presence of CECs has been also reported. Since these water bodies are the usual source for the production of drinking water, which is treated using target methods for removing specific organic pollutants, CECs can reach tap water.

CECs exhibit a wide range of polarity and chemical properties, rendering traditional extraction techniques, such as solid phase extraction or liquid-liquid extraction inefficient for the multi extraction of CECs. Furthermore, while traditional target approaches allow the detection and determination of known chemical compounds based on their correspondence with analytical standards, suspect and non-target approaches, mainly based on liquid chromatography high-resolution mass spectrometry (LC-HRMS), allow to detect and identify thousands of chemicals without standards.

Seeking to overcome the drawbacks of traditional approaches for extraction and determination of CECs, this research proposes the use of supramolecular solvents (SUPRAS) for the extraction of CECs from tap water and their analysis by LC-HRMS. For this purpose, tap water (n=53) from 12 countries around the world have been analyzed with the developed suspect screening workflow. Fifty-three CECs belonging to personal care products, stimulants, UV filters, surfactants, sebacates, industrial chemicals, lipids, plasticizers, cleaning products, and adhesives categories were identified in the analyzed samples. The concentration of chemicals identified with a level of confidence above 2b have been semi-quantified and a subsequent tentative evaluation of risks to human health has been performed. Some of the identified compounds showed values of risk quotient (RQ) above 1, the limit considered safe for humans, indicating a potential risk to human health. These results prove that the combination of SUPRAS and suspect screening analysis can be an effective strategy for discovering new contaminants and sources of exposure to humans.

### **3.03.P-We152 High Resolution Mass Spectrometry Solutions to the Challenge of Non-Target Transformation Product Identification Presented by the 2023 EFSA Drinking Water Guidance Document**

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The EFSA guidance document on the impact of water treatment processes on residues of active substances or their metabolites in water abstracted for the production of drinking water was issued in August 2023

(DOI: <https://doi.org/10.2903/j.efsa.2023.8194>). In general, the guidance covers the identification and exposure assessment of transformation products (TPs) of Biocidal and Plant Protection Products (PPPs) using experimental and *in silico* methods. This does not just cover the effects of water treatment on the active substances, but also on their transformation products along with an impact assessment covering the environment, human health, and domesticated animals.

Drinking water treatment is generally performed in several ways; the simplest being filtration (over activated carbon or sand) to absorb contaminants, to active breakdown with processes such as chlorination with sodium hypochlorite or chlorine, chlorination with chloramine, pre-oxidation with chlorine dioxide, ozonation, ultraviolet (UV) disinfection or a combination of several processes. The guidance provides methods for simple laboratory procedures which are intended to be performed without the need for complex and expensive equipment.

However, performing the laboratory tests is not the major challenge when considering this guidance. The number and complexity of potential transformation products can be great when considering the multiple processes involved. This undoubtedly will lead to extensive efforts to identify many transformation products creating the major challenge of non-target identification of potential TPs by LC-MS/MS or other structure elucidation techniques. This will likely be more challenging in the absence of any radiolabelled substances for active ingredients or their metabolites.

This presentation focusses on the strategies that can be employed using High Resolution Mass Spectrometry (HRAM) with (U)HPLC to perform non-target identification using available tools such as instrument automation and software interrogation on samples derived from water treatment processes.

### **3.03.P-We153 Stormwater Ponds as a Hotspot for Chemical Pollution and their Impact in Recipient Water Bodies and Drinking Water Productin**

*Victoria Eriksson, Oksana Golovko, Karin Wiberg and Alberto Celma, Swedish University of Agricultural Sciences (SLU), Sweden*

Street run-off stormwater is often polluted with a wide range of organic pollutants as a result of traffic, pest control, road wear, buildings and other human activities. Although most organic micropollutants occur in low concentrations, many of them are highly toxic and, thus, even low concentrations might threaten the environment by harming aquatic organisms. Also, human health can be affected since, in Sweden, the vast majority of drinking water is produced from surface water bodies.

In this work, we have investigated both influent and effluent water streams from a stormwater pond impacted by heavy traffic and residential and industrial areas. In addition, in case of a heavy rain event, the nearby pump station for municipal wastewater gets overloaded and direct discharge of untreated wastewater is diverted to the stormwater pond under study. To investigate the impact of rain in the fate of organic micropollutants in the pond and its implications, water samples were



collected both at dry periods (base flow) and rain events. Additionally, the outlet of the stormwater pond under study mouths in a large surface water body in Sweden, which serves a source for drinking water production for a large number of inhabitants. Thus, water samples were also collected in connection with heavy rain events at the inlet of a drinking water production facility a few kilometres downstream of the effluent of the stormwater pond.

Mixed-mode solid-phase extraction (SPE) and ultra-high pressure liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) were used for wide-scope screening of organic micropollutants of the samples permitting to expand the chemical coverage in comparison with conventional sample analysis strategies. Data revealed the presence of a large set of chemicals resulting from different human activities in both the influent and effluent streams from the stormwater pond. Yet, several of the identified compounds were also found in the inlet water sample of the drinking water production facility. In total, 59 organic micropollutants were identified, being vehicle related compounds and pharmaceuticals the most frequently detected compounds.

In this communication, we aim at presenting the analysis workflow as well as highlighting the most relevant aspects and implications of the identified compounds for the drinking water production.

### **3.03.P-We154 Mosaic-Project: Suspect Screening in 545 Water Bodies in Southern Germany**

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The monitoring-project MOSAIC intends to give a broad overview on the pollution status of Bavarian surface waters, with special focus on smaller rivers. Sampling sites were selected based on land use and potential point sources in the catchment area. A broad range from areas with high industrial density to rather agriculturally influenced sites is covered. 545 different water bodies were sampled quarterly and analysed by target analysis for a set of more than 240 parameters.

In addition to the parameters already addressed by target analysis, suspect screening is an upcoming and promising tool to identify further contaminants in the water samples. Samples were analysed by direct injection and LC-HRMS. Compounds were identified by MS/MS. A large set of more than 1000 compounds including pharmaceuticals, industry chemicals, pesticides and biocides as well as some metabolites was analysed by suspect screening.

To improve the quantitative information of suspect screening measurement, semi quantification of carbamazepine and terbuthryn was compared to target measurements in more than 700 samples. For samples with concentrations > 20 ng/L suspect screening and target measurements agreed well. Thus, semi quantification by internal standard provides the opportunity to roughly estimate concentrations of suspect compounds in surface water samples.

Suspect screening can additionally be used to provide a first estimate on the relevance of newly regulated compounds that are not addressed in standard monitoring programmes. For the revised WFD new compounds with EQS proposals are discussed. These were screened by suspect screenings in all 545 samples. Data show that the pharmaceuticals carbamazepine, diclofenac and sulfamethoxazole were detected in more than 50% of the investigated water bodies, whereas compounds such as the insecticide thiamethoxam and the antibiotic erythromycin were detected infrequently.

Screening a set of 37 common pharmaceuticals revealed, that ten of them were present in more than 66% of water bodies. The highest detection frequencies were determined for gabapentin, venlafaxine, carbamazepine, lamotrigine and metoprolol.

This project has the potential to gather a comprehensive overview of compounds present in smaller rivers and streams beyond just information on priority substances. Suspect screening strategies enable to get insight in the expected detection frequency of contaminants of emerging concern and compounds coming to regulatory interest.

### **3.03.P-We155 Suspect and non-target screening of legacy and emerging pollutants in the freshwater system of South Korea**

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This study investigated the occurrence, distribution, and bioaccumulation of various environmental pollutants, including both legacy and new persistent organic pollutants (POPs), as well as contaminants of emerging concern (CECs). The analysis was conducted through suspect and non-target screening (SNTS) using gas chromatography coupled to quadrupole time-of-flight mass spectrometry (GC-Q-TOF/MS) in both biotic and abiotic media of the freshwater system in South Korea. A total of 348 compounds, comprising 17 polychlorinated dibenzo-dioxins/furans (PCDD/Fs), 18 polychlorinated naphthalenes (PCNs), 62 polychlorinated biphenyls (PCBs), 41 polybrominated diphenyl ethers (PBDEs), 7 novel brominated flame retardants (NBFRs), 10 organophosphate flame retardants (OPFRs), 8 benzotriazole UV filters (BUVs), 8 volatile methylsiloxanes (VMSs), 27 plasticizers, 3 synthetic musk compounds (SMCs), 16 polycyclic aromatic hydrocarbons (PAHs), 17 phenols, and 114 pesticides, as well as unknown chemicals, were identified and qualified using an in-house database and the National Institute Standard and Technology (NIST) library, respectively. As a result of suspected screening using an in-house database,

a total of 93 compounds were identified at least once in the environmental matrix (river, sediment, and biota). The number of compounds identified in each group was 24 pesticides, 21 plasticizers, 15 PAHs, 10 phenols, 7 VMS, 3 SMCs, and 3 BUVs except for NBRFs, PBDEs, PCBs, and PCNs groups. When prioritizing pollutants based on detection frequency in river water, sediment, and crucian carp samples, 2-Methylphenol (o-cresol) emerged as the highest priority material, detected in 32 out of 47 samples. Evaluating the peak intensity of detected compounds in river water revealed that polychlorinated phenols (PCPs) accounted for 29-39% of the total combined peak intensity across all four water systems. In sediment, PCPs, the most dominant compound group, accounted for 37-67%. In carp, similar to other matrices, PCPs were the most dominant at 31-34%, followed by volatile organic compounds (VOCs) and PAHs. Detailed information of growth-dependent accumulation and contamination patterns of major contaminants will be presented during conference.

### **3.03.P-We156 Improving Nontarget Screening by Homologous Series Analysis: a Case-study in Swiss Sludge**

**Pablo Antonio Lara Martín<sup>1</sup>, Lena Schinkel<sup>2</sup>, Yves Eberhard<sup>2</sup>, Walter Giger<sup>3</sup>, Michael Berg<sup>2</sup> and Juliane Hollender<sup>4</sup>,**  
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Only a fraction of the potential chemical contaminants are nowadays regulated or regularly monitored in the environment. Advances in high resolution spectrometry (HRMS) allow for an easier and faster identification of new compounds with no previous selection of target analytes (nontarget screening, NTS). We have conducted a national survey in Switzerland to perform NTS of organic contaminants in sewage sludge, representing fingerprints of the corresponding wastewater and therefore population usage and exposure to chemical compounds. Identification of contaminants has been improved over previous works by relying not only on reference standards and MS/MS libraries, but also by conducting analysis of homologous series exploring different combinations of elemental compositions and repeating units to plot Kendrick mass defects plots. By performing online search in MS/MS spectral libraries and using available standards, up 78 contaminants could be identified at level 1 and semi-quantified including octocrylene, galaxolidone and triclosan. Samples from 3 WWTPs had a very distinctive chemical fingerprint compared to the rest (e.g., higher abundances of plasticizers), suggesting the existence of local pollution sources and/or different usage/consumption patterns in the areas served by the aforementioned WWTPs. NTS screening was completed through the identification of over 100 different series with more than 3 components each were annotated, spanning over a wide range of elemental compositions: CHO (n = 66, e.g., polyethylene glycols), CHN (n = 12, e.g., C6-C34 alkylamines), CHNO (n = 28, e.g., alkylbetaines), CHSO (n = 12, e.g., linear alkylbenzene sulfonates), CHPO (n = 4, e.g., trialkyl phosphates), CHNSO (n = 4, e.g., alkyl isothiazolinones), CFO (C6-C12 perfluoroalkyl acids), and CFSO (C4-C9 perfluoroalkyl sulfonic acids). Most of the identified chemicals were either naturally occurring products (e.g., fatty acids and other cellular components) or high production volume chemicals such as synthetic surfactants and polymers. Homologous series analysis allowed to establish links among different features that were overlooked by the preliminary and more conventional NTS approach only relying on MS/MS libraries, analytical standards and prioritization of features through statistical analyses, resulting in a 4-fold increase in the number of tentatively identified compounds, and therefore allowing to explore the acquired data in a more comprehensive way.

### **3.03.P-We157 What are Contaminants of Emerging Concern (CECs) in Swedish Landfill Leachate?**

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Landfills are the destination of much unrecyclable waste, but they are not the final destination for contaminants. Contaminants of emerging concern (CECs) are harmful organic chemicals used extensively in consumer and industrial products that can eventually enter the environment through landfills. Before Sweden introduced stricter regulation on waste management, a wide variety of residues was deposited in landfills, underscoring the importance of investigating the occurrence and levels of CECs in order to fully understand their ecological and human health impacts. Non-target screening (NTS) and suspect screening (SS) by Ultra High Performance Liquid Chromatography-Electrospray Ionization- High Resolution Mass Spectrometry (UHPLC-ESI-HRMS) are widely used strategies for identifying unknown compounds in samples. The goal for this study is to create a list of representative CECs in Swedish landfill leachate for future monitoring and quantification.

In our study, samples (untreated and treated leachate) were collected from five different Swedish landfills with the variations of different locations, age, and treatment methods. Both individual samples and pooled samples were prepared before analysis. Given the high sensitivity of the UHPLC-ESI-Orbitrap instrument, analysis of pooled samples addresses the common challenges of extensive data processing required for individual analysis. Extraction was performed on homemade multi-layer Solid Phase Extraction (SPE) cartridges in order to capture a wide spectrum of CECs. Preliminarily, pharmaceuticals, personal care products, industrial chemicals, and Per- and Polyfluorinated Substances (PFAS) were identified in the landfill leachates. Overall, our study helps to bridge the knowledge gap regarding the occurrence of CECs in Swedish landfills and lays the groundwork for future monitoring and regulatory efforts.

### **3.03.P-We158 Exploring Non-Target Screening and Differential Analysis in Assessing Nature-Based Reactive Barrier Treatment of Micropollutants in Treated Wastewater**

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Population growth and increasing water demand have exacerbated water resource scarcity. Treated wastewater (TWW) is a valuable alternative resource, but its complex composition, including micropollutants, raises concerns about reuse risks. Reactive barriers (RB) made with natural materials offer a solution to remove TWW contaminants. Evaluating RB process effectiveness and comprehending contaminant fate remain challenging. Recent advances in High Pressure Liquid Chromatography (HPLC) coupled with High-Resolution Mass Spectrometry (HRMS) and non-target analysis (NTA) provide an opportunity to identify contaminants and evaluate RB processes. The study aims to develop an NTA approach to assess RB effectiveness and understand the quality of water treated with RB concerning emerging contaminants (ECs).

In a series of batch experiments, TWW was mixed with a RB under two conditions: TWW in contact with RB (TWW+RB) and TWW alone (TWW). After one hour of contact, samples were extracted and analyzed using HPLC-HRMS. NTA workflow and statistical comparison between the two sample conditions were applied for data treatment. Additionally, a quantitative method was applied to 28 pharmaceutical residues. Sorption (%) was calculated by comparing concentrations in TWW+RB and TWW experiments. NTA's applicability for sorption estimation was investigated using intensities from differential analysis.

Differential analysis shows significant differences in compounds that decrease or increase after contact with RB. A prioritization workflow identified and annotated 115 compounds. Natural products predominantly increased after RB contact, while compounds exhibiting a significant 75% decrease in peak area are mainly pharmaceuticals. Among the 28 quantified pharmaceuticals, 18 were detected in the TWW influent, with concentrations ranging from less than 10 ng/L to over 1 µg/L. Notably, 61% of these molecules were sorbed at more than 50%. The sorption of ECs estimated by NTA correlated satisfactorily with sorption quantified through target analysis, although caution regarding the matrix effect is necessary. NTA provides a comprehensive view of RB treatment performance, giving information on the compounds released by the barrier and its sorption capacity. NTA emphasizes the benefits of RB treatment while highlighting the need to study compounds reloaded by RB. It is a powerful tool for understanding the advantages of nature-based reactive barrier treatment in improving the quality of TWW.

### 3.03.P-We159 Quaternary Ammonium Compounds in Produced Water Wastewater

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Produced water (PW) is a significant byproduct of oil and gas production, sometimes exceeding the hydrocarbon production in volume, and it constitutes a major wastewater stream in the Danish North Sea offshore production. While existing regulations address dispersed hydrocarbon content, there are currently no limiting regulations addressing other potentially hazardous compounds, including production-related chemicals such as biocides, hydrogen sulfide scavengers and scaling inhibitors added during the oil production process. Quaternary ammonium compounds (QACs) are a class of cationic surfactants, they have anti-fouling and biocidal properties and they are of concern for the environment, since there is a lack of data about the concentration range and degradation of these compounds in the offshore waste stream. In this study quaternary ammonium compounds benzododecinium, benzyldimethyltetradecylammonium, didecyldimonium, benzyldimethylhexadecylammonium are investigated together with non-target analysis of production chemicals on PW wastewater from Danish offshore production platforms. The analysis is performed by SPE extraction and subsequent analysis by LC high-resolution MS using an electrospray ionization source. This study can help to better understand the quality of the offshore wastewater and provide input to environmental impact assessment and mitigation strategies to reduce the overdosing of production chemicals in hydrocarbon production.

### 3.03.P-We160 Scaling up Suspect Screening Analysis (SSA) in environmental water samples: a case study of contaminants of emerging concern in wastewater

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Contaminants of emerging concern (CECs) are increasingly being shown to occur in water samples across the world. Consequently there is a constant need for reliable analytical methods for identification and determination at low levels (ng/L-µg/L). Sample pre-treatment (e.g., solid-phase extraction) is widely applied to reach the required sensitivity but is usually time-consuming. New direct-injection methodologies have emerged which combined with liquid chromatography-high resolution mass spectrometry (LC-HRMS) offers enough sensitivity to rapidly identify large numbers of chemicals. The aim of this study is to monitor a wide number of CECs using direct injection suspect screening analysis (SSA) in a national wastewater monitoring campaign.

Influent 24h-composite samples were collected across 18 wastewater treatment plants in England (2021-2023). Samples were only filtered using 0.2 µm PTFE filters and transferred to LC amber vials for storage and/or analysis. SSA was performed using a Shimadzu LCMS-9030 LC-QTOF-MS instrument, a Shim-pack Velox 2.1 × 100 mm, 2.7 µm biphenyl column, a 17 min LC gradient and 40 µL of injection volume. ESI-HRMS was run in positive and negative ion mode, and fragmentation was performed in data independent analysis. Shimadzu LabSolutions Insight Explorer Library Screening software containing ~1295 compounds, and four points of confirmation were used: ±0.5 min retention time, ±5 ppm mass accuracy, isotopic distribution

score >20 and a library identification similarity index score >45. In addition, the signal-to-noise ratio was >3:1 and a >1,000 peak intensity threshold was used.

A total of 140 samples were analysed, where 292 unique compounds were matched to library data. Drugs showed the highest number of compounds detected including antidepressants. Confidence levels were assigned following the Schymanski framework. To investigate a spatial comparison between sites, an investigation of stable isotope labelled internal standards (SIL-IS) stability between batches and replicates was performed. A selection of SIL-IS was investigated to use their peak height for normalisation to achieve a hierarchical cluster analysis across all samples. For the first time, direct-injection SSA was successfully applied as a high-throughput technique to a national wastewater sampling campaign. This work presents the first investigation of a high spatiotemporal wastewater analysis across England (~28 % of the country's population).

### **3.03.P-We161 Suspect Screening Reveals the Presence of Ultra Short Chain PFAS in Australian environmental Water Systems**

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The class of Per- and polyfluoroalkyl substances (PFAS) comprises hundreds of thousands of chemical compounds, with many causing significant environmental contamination. Recent regulatory changes have pushed industries to seek alternatives with shorter carbon chains, like short-chain fluorinated options (C4-7) and ultra-short chain products (C≤3). While there's been considerable focus on tracking both short and long-chain PFAS (C>4), Ultra Short Chain (USC) PFAS have been less explored due to analysis difficulties. Consequently, gaps persist in understanding how USC PFAS spread in the environment and the associated risks.

This research aims to fill this void by studying the presence and destiny of USC PFAS in different environments across Australia—groundwater, surface water, wastewater, and drinking water sources. The study involves creating a new High-Resolution Mass Spectrometry suspect screening method combined with a targeted LC-MS/MS method. Our investigation identified fourteen distinct USC PFAS compounds, including eight not previously reported in Australia, such as PFPrA, PFMeS, PFETs, PFPrS, MeFMeSA, FPrSA, H-PFETs, H-PFPrS, Cl-PFPrS, H-PFPrA, PFPrSi, PFPr-OS, and NTf2.

Although toxicity data for USC PFAS are scarce, their widespread presence in the environment, especially PFPrS at higher concentrations, emphasizes the need for improved monitoring efforts. Analyzing trifluoroacetic acid (TFA) remains challenging due to background levels and contamination, requiring further research and method refinement. Overall, this study offers critical insights into the prevalence and behaviour of USC PFAS, emphasizing the urgency for continued research and regulatory focus on this emerging contaminant class.

### **3.04.A Analysis, Assessment and Management of Contaminants of Emerging Concern and Their Transformation Products in the Environment**

#### **3.04.A.T-01 Per- and Poly-fluorinated alkyl substances (PFAS) at the Environment Agency UK**

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Per- and Poly-fluorinated alkyl substances (PFAS) are a group of widely used synthetic substances. They are extremely persistent in the environment and as a result they have been dubbed the “Forever Chemicals”. This along with their widespread use means many are now ubiquitous in our environment

Source site screening work has been undertaken to assess the risk from PFAS emission to the environment for over 10000 potential sites across the UK that considers the site types and environmental setting. This work has been used to produce risk maps that inform monitoring strategies and engagement strategies. Recent monitoring undertaken across England has shown how PFAS are detected in >65% of surface water samples, >20% of groundwaters and in all saline and freshwater fish analysed. Biota analysis has also shown how PFAS are now ubiquitous in freshwater and saline fish from around the country. While PFOS still remains the dominant PFAS detected in fish samples, the presence of those shorter chain PFAS used as replacements are such as PFBA, PFPeA, PFBS and 6:2 FTSA are found in water samples. We are also using a number of emerging analytical methods such as TOP Assay and Passive sampling to improve our understanding of PFAS and reduce monitoring costs.

Investigations into high risk sectors such as contaminated land, landfills waste water treatment works and manufacturing sites is helping to build a detailed picture of PFAS sources and identify those that pose the highest risk to human and environmental health.

Our work has shown how PFAS are being detected across England's environment however the data also shows how 19 of the PFAS included in our monitoring suite of 45 PFAS are not being detected. Data is also showing how many areas of the country

are not impacted by PFAS with reported concentrations being below the limit of quantification. By presenting the data in a clear manner we hope to provide an accurate, unbiased representation of PFAS in the English environment.

#### **3.04.A.T-02 Background concentrations and spatial distribution of per- and polyfluoroalkyl substances (PFAS) in surface waters of the greater Melbourne area, Australia**

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Little is known about the background concentrations of per- and polyfluoroalkyl substances (PFAS) in Australian urban waterways and whether their presence and concentrations are associated with particular catchment land-uses.

A survey was conducted of 33 per- and polyfluoroalkyl substances (PFAS) in streams and wetlands (n = 65) in Greater Melbourne, Australia, to determine background concentrations and major catchment land-uses contributing to PFAS pollution. Sites consisted of streams and constructed urban wetlands with independent catchments that were classified by their dominant catchment land use – either residential, industrial, municipal wastewater treatment plants, or rural. Melbourne was considered a suitable city to establish background concentrations as there is no manufacturing of PFAS and few point sources of PFAS pollution. Surface water and samples collected from 65 sites were analysed for thirty-three PFAS.

Twenty-two out of thirty-three targeted PFAS were detected, with at least one PFAS species was detected in 98% water samples. While diffuse PFAS pollution was detected across the urban waterways, significant differences between land-use types were observed depending on the PFAS congener and also on the PFAS class (perfluorosulfonic acids (PFSAs) or perfluorocarboxylic acids (PFCAs)). Results suggests that different types of PFAS-containing products were used in the various catchment land-uses and that congeners transported diffused through air and water to varying degrees. Results will be discussed based on occurrence patterns across the different land-use types for total PFAS and individual PFAS compounds.

#### **3.04.A.T-03 Increasing Atmospheric Depositions of Trifluoroacetate (TFA) Over the Last Three Decades**

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Trifluoroacetate (TFA) is a very persistent and very mobile (vPvM) contaminant that occurs ubiquitously in the environment. Among many anthropogenic sources, TFA can be formed in the atmosphere through the oxidation of certain perfluoroalkyl-containing substances, such as hydrofluorocarbons (HFCs), hydrochlorofluorocarbons (HCFCs), hydrofluoroolefins (HFOs), and hydrochlorofluoroolefins (HCFOs), which were introduced as substitutes for ozone-depleting chlorofluorocarbons (CFCs) and find wide application as refrigerants, fire extinguishing agents, and physical blowing agents. The usage of numerous precursors has considerably increased over the last decades. Therefore, with increasing emissions of these compounds, the TFA levels in precipitation are expected to rise. In this study, archived plant leaf samples from the German Environmental Specimen Bank (ESB) were used as a biomonitoring tool to give insights into the temporal trends in the atmospheric depositions of TFA in Germany over the last three decades. The TFA concentrations of investigated deciduous and coniferous tree leaf samples mostly ranged from tens to hundreds of  $\mu\text{g}/\text{kg}$  based on dry weight. With the exception of one site, significant ( $p < 0.05$ ; Mann-Kendall tests) positive temporal trends in TFA concentration were found for all of the tree species. The uptake and transpiration of water by vascular plants to replace the water lost due to photosynthesis provides a mechanism for bioaccumulation of TFA. Consequently, increasing levels of TFA in the studied perennial plants are likely a product of both phytoaccumulation over multiple years and an upsurge in the anthropogenic emissions of gaseous TFA precursors, leading to an increasing atmospheric deposition of TFA in the Northern Hemisphere over the last three decades. This study has shown that the analysis of plant matrices can be an efficient biomonitoring tool to evaluate the temporal and spatial presence of TFA in the terrestrial environment. It is expected that the atmospheric formation and deposition of TFA will continue to grow in the coming years mainly due to rising global emissions of fluorinated refrigerants and the switch to the latest generation of refrigerants, namely HFOs, as they generally degrade faster and with larger TFA yields than their predecessors.

#### **3.04.A.T-04 Formation of trifluoroacetic acid from the aqueous photolysis of aryl-CF<sub>3</sub> compounds**

**Shira Joudan, Zhefei Guo and Azka Attar, Department of Chemistry, University of Alberta, Canada**

Concentrations of trifluoroacetic acid (TFA) in the environment exceed the expected concentrations based on emissions of TFA and its known precursors. In 2020, 54 pharmaceuticals and 117 agrochemicals had aryl-CF<sub>3</sub> functionalities, and there are reports of TFA forming from some of these molecules. Limitations of previous research arise from not monitoring all transformation products throughout the experiment leaving uncertainty in reaction mechanisms and some miscalculated kinetics. A common aryl-CF<sub>3</sub> motif is para-hydroxybenzotrifluoride (p-OHBF) including in the pharmaceutical fluoxetine and the pesticide difenopent. The objective of this work was to test the photochemical reaction of p-OHBF to determine kinetics, quantum yield and product formation with an emphasis on TFA vs F<sup>-</sup>. Photochemistry experiments of p-OHBF were performed in quartz test tubes using a photoreactor with UVB lights and dark controls were performed in the same tubes. p-OHBF and its carboxylic acid reaction product were quantified using HPLC-UV, while TFA and F<sup>-</sup> were quantified using ion chromatography with conductivity detection. The formation of other transformation products were explored via <sup>19</sup>F NMR and non-target mass spectrometry. The direct photochemistry of p-OHBF was accelerated at higher pH and slower at lower pH, confirming previous reports. Rate constants were 0.016 h<sup>-1</sup> (pH 4.3, quantum yield 0.17), 0.012 h<sup>-1</sup> (pH 7.4, quantum yield 8.1

$\times 10^{-3}$ ), and  $0.0097 \text{ h}^{-1}$  (pH 8.2, quantum yield  $1.2 \times 10^{-3}$ ). Base-catalyzed defluorination of p-OHBF was observed in the dark to form the carboxylic acid and  $\text{F}^-$  at pH 7.4 and 8.2, but did not occur at pH 4.3. Kinetic analysis determined the maximum TFA formation at pH 4.3 is 9.32%, at pH 7.4 is 3.49% while at pH 8.2 is 1.89%. This shows that especially at acidic conditions, p-OHBF can be converted to nearly 10% TFA, and not solely undergo defluorination. TFA was only observed in photochemistry experiments, while defluorination is primarily a base catalyzed reaction that is also relevant at pH 7.4. All experiments have fluorine mass balance greater than 90%. Overall, if up to 10% of an Ar- $\text{CF}_3$  molecule is converted to TFA from direct photolysis only, this may add up to a large source in sunlight waters when accounting for other formation pathways such as indirect photolysis or biotransformation. This workflow is a tool that can be applied systematically to Ar- $\text{CF}_3$  molecules to assess their contributions to the presence of TFA in the environment.

### **3.04.B Analysis, Assessment and Management of Contaminants of Emerging Concern and Their Transformation Products in the Environment**

#### **3.04.B.T-01 IN SILICO TENTATIVE IDENTIFICATION OF PHARMACEUTICAL BIOTRANSFORMATION PRODUCTS IN RECEIVING WATER**

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Water and effluent quality monitoring methods in the UK are predominantly focused on organic matter (nitrogen, phosphorus levels) whereas pharmaceuticals and their biotransformation products (BPs) are not monitored routinely in river water. Antimicrobials such as sulfamethoxazole and clotrimazole are pharmaceuticals that are of particular concern, increasingly being detected in surface water and soils across Europe, are prone to causing antimicrobial resistance and are toxic and mobile in the aquatic environment.

This work aims to fill a research gap in the lack of predictive understanding of pharmaceutical transformation and degradation; high resolution analytical methods have been developed for in silico identification of pharmaceuticals and their BPs but do not provide information about the mechanisms or pathways of transformation. It will determine the biotransformation rate of prioritised compounds (including those in the EU WFD “watch list”) and investigate their transformation pathways to help gain a better understanding of their breakdown in river water. The microbial and chemical data derived from these studies will be used to create a machine learning based model that can help predict the biotransformation of pharmaceuticals in river water.

Suspect screening in high resolution mass spectrometry (HRMS) analysis has been used to detect and identify compounds and 900 BPs in wastewater impacted rivers and the use of online predictive transformation tools such as BioTransformer 3.0 (BT 3.0) and EAWAG-Biocatalysis/Biodegradation Database/ Pathway Prediction System (EAWAG—BBD/PPS) have been used to predict BPs that could be formed in environmental microbial transformation (EMT). Compounds of interest were prioritised on the basis of occurrence (frequency across sites and peak height) and risk quotients (RQs), resulting in a shortlist that includes carbamazepine, lamotrigine and venlafaxine. Stability studies are being conducted them in riverwater with the use of LC-MS/MS and HRMS analysis.

Future work will include the application of the retention time model to other pharmaceuticals in order to identify other BPs in HRMS data, as well as assessing identified BPs in terms of environmental risk e.g. predicted no effect concentration (PNEC). Biological analytical methods (metagenomics, metatranscriptomics, qPCR) will also be conducted to observe the resident enzymes and/or microbial communities that may be involved in the observed transformations.

#### **3.04.B.T-02 Computational Assessment of Antibiotic Transformation Product Activity: Molecular Dynamics Simulations and Free Energy Calculations**

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The global proliferation of antimicrobial resistance (AMR) poses a significant threat to human, animal, and the environmental health within the concept of One Health. Current evaluations of AMR and associated environmental risks predominantly focus on antimicrobial parent compounds, often neglecting their transformation products (TPs). Being generated through organismal excretion and/or transformed during wastewater treatment, TPs frequently emerge through subtle alterations in molecular structure, often resulting in increased stability and persistence, ultimately infiltrating surface water ecosystems. Given the structural similarity between TPs and their parent compounds, the potential interaction of TPs with the target protein of their parent compounds remains a critical concern for the emergence of AMR genes in the environment. This study used the open-source molecular dynamics software, GROMACS, together with PyAutoFEP for free energy calculations to assess the binding affinity differences to the respective target protein between TPs and their parent compounds. We explored several sulfonamide TPs, as well as TPs from trimethoprim and multiple antivirals, like nevirapine, lamivudine, or acyclovir. Many TPs exhibited predicted binding affinities within the similar range as the parent compound, with some showing increased affinity, suggesting potential activity. This research highlights the importance of expanding our approach for AMR assessment by recognizing the significance of antimicrobial transformation products.

### **3.04.B.T-03 Pesticide transformation product occurrences in surface waters as ground water pollution risk indicators in the context of an extended residence time aquifer**

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Luxembourgish groundwater aquifers have recently been impacted by high concentrations of several pesticide transformation products (TP) that led to regulatory issues for about 1/3 of the drinking water supplies (Luxembourg considers all transformation products to be relevant and applies the 100 ng/L legal threshold). The consequent application restrictions or complete bans of certain pesticides triggered switches in compound uses in several cultures. A leaching risk analysis had been conducted to orient agricultural counseling on the least impacting parent compounds and transformation products. However, since the leaching simulations relied on literature environmental property data of the pesticides (like fraction of TP generated), there was some uncertainty on the true impact of the identified TPs. Residence times of ground waters in the main aquifer spanning on average between 10 and 20 years (with proportional recovery times once these waters are contaminated), a faster validation approach was needed. The hypothesis was established that the interflow component in surface waters would be a good indicator to estimate the amount of transformation products available for groundwater leaching. In that perspective passive sampling campaigns were established on four river basins of distinct hydrogeology to quantify the masses of parent compounds and transformation products transported during an entire year and supported by grab sampling in the descending limbs of seasonal flood waves. Additional parameters included conductivity, DOC, Abs280 and macro-anions. All the predicted transformation products were identified and their occurrence varied in amount and timing in the different catchments according to their application, metabolization in soils and hydrogeological setting. This contribution discusses the influences of spatial use variability and hydrological connectivity of agricultural source plots to the magnitude of the occurrences as well as its potential link to the leaching modelling and its parametrization.

### **3.04.B.T-04 Identification of Neonicotinoid Transformation Products in Aquatic Environments**

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Despite their increasing scrutiny and discontinued use in several countries, neonicotinoid insecticides are still widely used in Australia, with over 400 neonicotinoid-containing products registered for use. The aquatic environment is a major sink of neonicotinoid insecticides from sources such as agricultural runoff, and wastewater treatment plant effluents released into environmental waters, which may put aquatic species at increased risk of harm from neonicotinoid exposure.

Photodegradation is an important factor in the environmental fate of aquatic contaminants and is the primary process of environmental degradation of neonicotinoids in aquatic environments. Several studies have investigated neonicotinoid degradation kinetics, with findings suggesting all neonicotinoids follow first-order or pseudo first-order kinetic degradation; and are preferential toward transformation over mineralisation. However, little is known about transformation pathways, the structures of the final stable transformation products, or the risk of these transformation products to the aquatic environment.

The aims of this study are to identify environmentally relevant transformation products and their transformation pathways from four commonly used neonicotinoids (i.e., clothianidin, dinotefuran, imidacloprid and thiamethoxam) and assess risk of the identified transformation products to the aquatic environment.

Neonicotinoids from analytical standards and commercial formulations were exposed to UV radiation over a time course from 0-24 hours. Neonicotinoid parent compounds were quantified at various time points to investigate degradation kinetics. Samples were analysed by high resolution mass spectrometry using both suspect screening and non-target workflows to identify transformation products and elucidate transformation pathways. Finally, the US EPA's Toxicity Estimation Software Tool was used to investigate *in silico* toxicity of the identified transformation products to investigate the potential risk to aquatic environments.

Results indicate that each of the studied neonicotinoid parent compounds degrade to 3-4 environmentally relevant transformation products upon UV exposure, which we were able to elucidate transformation pathways for. Several of the identified transformation products are estimated to be of greater toxicity to aquatic organisms compared to parent compounds, suggesting neonicotinoids pose a greater threat to the aquatic environment than previously perceived.

### **3.04.C Analysis, Assessment and Management of Contaminants of Emerging Concern and Their Transformation Products in the Environment**

#### **3.04.C.T-01 Advancing Surface Water Monitoring: A Comprehensive Strategy Integrating Tailored Pesticide Screens and Advanced Analytical Techniques**

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The continuous introduction of new pesticides globally poses challenges for effective environmental monitoring. This study proposes an innovative approach to identify priority pesticides for regional waterway monitoring. The method involves leveraging local pesticide registration data, assessing high toxicity to non-target aquatic organisms, and considering pesticides typically excluded from routine laboratory screening. To detect these priority pesticides in surface waters, a suspect screening analytical approach was developed, utilizing two passive sampling methods and high-resolution mass spectrometry (HRMS). The investigation focused on 181 priority pesticides across 32 waterway sites within Greater Melbourne, Victoria Australia, encompassing diverse catchment land uses. Liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) in data-independent acquisition mode (DIA) was employed for pesticide detection. Through a suspect screening workflow using Waters UNIFI software, 21 pesticides were tentatively detected at 22 sites, with confirmation of 5 pesticides using certified reference materials (CRMs). Notably, the study identified several newly emerging pesticides in Australian surface waters, previously undocumented. Confirming priority pesticides before their inclusion in routine screening enhances the efficiency and cost-effectiveness of monitoring programs. However, practical, and analytical limitations make confirming the presence of numerous compounds challenging. This study demonstrates the effectiveness of a regional screening approach, combining broad-field sampling and suspect screening through HRMS. This comprehensive strategy enhances understanding of emerging pesticide levels in specific regions, aiding in the prioritization of pesticides for routine screening programs.

### 3.04.C.T-02 A New Method for Detecting and Quantifying Phthalate Exposure Using Blubber from Bottlenose Dolphins (*Tursiops truncatus*)

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Phthalates are plasticizing chemicals added to a variety of consumer products. They are considered endocrine disruptors, which may adversely impact mammalian development, reproduction, and metabolism. Phthalates are not permanently bound to the products to which they are added, increasing the likelihood of environmental exposure for humans and wildlife. Recent studies in bottlenose dolphins (*Tursiops truncatus*) demonstrated prevalent exposure to phthalates, but current detection methods rely on urine samples collected during catch-and-release health assessments. These events are logistically complicated, expensive, and require animals to be handled. This study developed a protocol for phthalate ester metabolite (mPAE) detection in bottlenose dolphin blubber, a type of sample that can be collected remotely. We used solid phase extraction and high-performance liquid chromatography-tandem mass spectrometry to analyze blubber samples (n=27) collected during health assessments conducted in Sarasota Bay, FL, USA in 2016, 2017, 2022, and 2023. Detectable concentrations of three mPAEs (mono-2-ethylhexyl phthalate (MEHP), monoethyl phthalate (MEP), mono-isodecyl phthalate (MIDP)) were observed in 37% of sampled dolphins (n=27). The most commonly detected mPAEs included mono-2-ethylhexyl phthalate (MEHP; n=6; geometric mean: 4.57 ng/g wet weight; range: 5.2 to 12.4 ng/g ww), monoethyl phthalate (MEP; n=2; geometric mean: 44.9 ng/g ww; range: 39.7 to 50.1 ng/g ww), and mono-isodecyl phthalate (MIDP; n=3; range: 26.0-27.4 ng/g ww). These findings from blubber correspond with observations from urine in which measurement of MEHP and MEP was also most common. mPAE detection in blubber offers another surveillance tool that does not have the limitations of catch-and-release health assessments. Blubber can be obtained via remote biopsy methods, which are used globally to monitor marine mammal populations, thereby facilitating exposure assessments in other at-risk populations.

### 3.04.C.T-03 What is in our electrical and electronic plastic waste? Non target and target screening approaches for the identification and determination of hazardous substances

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Waste of electrical and electronic equipment (WEEE) remains as one of the fastest growing global wastes, mainly fuelled by rapid technological advances and accelerated obsolescence. This residue contains both valuable and hazardous substances, which difficult their recycling in an environmentally sound way. Recently, the European Commission presented a new circular economy action plan that has WEEE reduction as one of its priorities. To date, recycling to recover reusable components as Cu and precious metals from WEEE is the goal. However, due to the limited number of facilities and high costs, landfilling, incineration, and exportation to other countries remain as the main alternatives, posing environmental and human risks due to the presence of toxic substances.

Plastics constitute 20% of WEEE (WEEEP), which contain high concentrations of additives to prolong lifespan. The main limitation to achieving an actual sustainable circular economy is the lack of information regarding the chemical additives used in the different plastics and applications. The presence of hazardous organic compounds (HOCs) in the plastic waste of these devices carries profound environmental implications. Notably, HOCs like brominated flame retardants (BFRs), polychlorinated



biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) are of paramount concern. These hazardous compounds, together with other unidentified substances, arising from the additives used and from the physicochemical transformations during plastic treatment or production, further complicate the recycling of WEEEP.

Herein, a non-target screening analysis of WEEEP was carried out to identify volatile and semi-volatile additives using gas chromatography coupled to mass spectrometry (GC-QTOF-MS). More than 300 substances were tentatively identified, belonging to different families commonly used as plastic additives, flame retardants, PAHs, plasticizers, and UV filters. The semi-quantification of predominant additives was conducted across 48 WEEEP samples collected from different handling and dismantling facilities. Tetrabromobisphenol A (TBBPA) and triphenyl phosphate (TPhP) were the substances detected in the highest concentrations ranging from 14.0 to 7,292  $\mu\text{g g}^{-1}$ .

### **3.04.C.T-04 Spatial Distributions, Seasonality, and Ecological Risk Assessment of UV Absorbents in the Habitat of Endangered St. Lawrence Estuary Beluga in Canada**

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St. Lawrence (SL) Estuary beluga whales (*Delphinapterus leucas*) are endangered cetaceans population in Canada. Exposure to various contaminants is a major stressor affecting the health and recovery of this whale population. Nonetheless, as important factors influencing exposure, the prevalence and distribution of many emerging contaminants in the water of their habitat are poorly understood. UV absorbents (UVAs) are derived from sunscreens, personal care products, plastics, and other products to protect skin from sunburn or prevent the materials from UV-induced color change or degradation. UVAs have been detected in the tissues of SL Estuary beluga whales and their food web, demonstrating the presence of UVAs in the habitat of SL Estuary beluga and the potential input of these contaminants from the rivers draining into this system, such as the SL River and the Saguenay River. This study aims (i) to investigate the distribution and seasonality of two groups of UVAs, UV filters (UVFs) and benzotriazole UV stabilizers (BZT-UVs), in the dissolved phase and the suspended particulate matter (SPM) of the surface water in the habitat of the SLE beluga, and (ii) to conduct an ecological risk assessment of these contaminants in the SL surface water. Water samples were collected from 60 locations in the beluga habitat (the SL Estuary, SL River, and Saguenay River; 2019-2020) (Quebec, Canada) to conduct the spatial distribution study. In addition, between April and October 2023, water samples were collected from 3 sites in the SL Estuary for the seasonality study. UVFs concentrations (3-1570 ng/L) were generally greater than BZT-UVs (<Method Detection Limit (MDL)-43 ng/L) in the water samples. Compared to the SL Estuary, the river samples showed higher detection frequency and concentrations of four BZT-UVs and three UVFs in the dissolved phase. High abundances of UVAs on SPM were found in the SL, suggesting that the ingestion of SPM could be an important exposure pathway of aquatic organisms in the beluga habitat to BZT-UVs and UVFs. The seasonality study is ongoing, and an ecological risk assessment analysis will be presented. This study establishes a baseline for monitoring UVAs in St. Lawrence beluga whales' habitat.

### **3.04.D Analysis, Assessment and Management of Contaminants of Emerging Concern and Their Transformation Products in the Environment**

#### **3.04.D.T-01 Contaminants of Emerging Concern in Urban Stormwater in the San Francisco Bay Area**

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An urban estuary that supports a population of over 7 million people, San Francisco Bay (California, USA) serves as a useful laboratory for studies of anthropogenic contaminants in urban stormwater and impacts on receiving waters. The majority of the region is served by separate storm drain systems, which channel stormwater flows directly to the Bay without treatment. Although small local tributaries make up only 6% of the freshwater entering the Bay, they contribute a disproportionately large percentage of the pollutant load for many legacy pollutants of concern (e.g., polychlorinated biphenyls, dioxins, polycyclic aromatic hydrocarbons, trace metals, and pesticides). However, far less is known about the role of urban stormwater in delivering contaminants of emerging concern (CECs) to the Bay, a data gap generally observed for receiving waters around the world. To begin to address this data gap, the Regional Monitoring Program for Water Quality in San Francisco Bay has conducted a four-year screening study to evaluate the concentrations of key classes of CECs in stormwater. Sampling sites have been selected primarily based on the extent of upstream urban land uses, with an emphasis on proximity to roadways. Complementary sampling occurred in less urban reference watersheds, at near-shore estuarine sites with tidal mixing, and in the open Bay to evaluate contaminant occurrence and inform our understanding of contaminant transport pathways into estuarine waters. Four classes of compounds were monitored using a suite of liquid chromatography tandem mass spectrometry (LC-MS/MS) methods, including per- and polyfluoroalkyl substances (PFAS), organophosphate esters, bisphenols, and several chemicals associated specifically with urban stormwater, including vehicle tire ingredients and the transformation product N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPDQ). Results from this broad screening study will be

summarized. Findings are already informing the development of a sustained stormwater CECs monitoring and modeling approach for San Francisco Bay.

#### **3.04.D.T-02 Ecotoxicological risk assessment (ERA) of pesticides transformation products occurring in small waterbodies: case of ponds.**

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In Europe, particularly in France, many small waterbodies (SWB) are scattered throughout the hydrographic network but are not included in the monitoring networks of the Water Framework Directive (WFD), making them understudied areas. Ponds constitute a portion of these SWB and serve as valuable wetlands that provides both ecosystem and economic services. Due to their closeness to agricultural areas, they could exergue fluctuating contamination state, particularly in terms of Plant Protection Products (PPPs). The increased occurrence of PPPs and their residual forms (i.e. transformation products (TPs)) in wetland ecosystems has required the inclusion of these substances in water surface contaminant monitoring. There is a growing awareness concerning those products for which little is known about, especially in terms of occurrence and ecotoxicity.

Our focus lies in detecting these TPs across various ponds to gather data on their presence and subsequently identify potential ecotoxicological effects associated with them. This study's double objective is to establish pollution profiles of ponds (n=12) through chemical analysis, targeting 86 molecules and then to conduct an ecotoxicological risk assessment (ERA). This assessment was based on the measured concentrations (MEC) of PPP detected in ponds. Furthermore, ecotoxicological data were compiled, combining multiple data sources, in order to calculate a risk quotient (RQ) and identify TPs that could be harmful for this specific aquatic environment.

Concerning the occurrence, each pond displays varying composition and levels of PPP and TP. It has been quantified from 2 to 21 molecules among the ponds, going from  $4,7 \pm 0,2$  to  $648,5 \pm 33,5$  ng/L. TPs from current and banned pesticides were detected, like flufenacet-oxa and 2-hydroxyatrazine. Flufenacet-esa and MCPA were found at the  $\mu\text{g/L}$  level in the most polluted pond. Then, following the ERA, the pesticide risk was mostly driven by herbicides from the triazines group.

This study demonstrates that the risk depends on both the diversity of molecules present in the system under consideration and the concentration levels that may constitute a hazard to the wildlife reliant on these environments. The simultaneous presence of multiple pesticides might elevate the risk for aquatic organisms across all the sampled locations. This work brings newest data concerning pesticide risk in a little-studied environment with an emphasis on the transformation products.

#### **3.04.D.T-03 Grouping persistent and mobile substances based on persistent and mobile substructures to avoid regrettable substitution and prioritize assessments**

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There is an interest by regulators and scientists working with chemical hazard assessment to transition from a one-substance-at-a-time approach to a substance-group approach. Two main motivations for this are to expedite hazard assessments related to the large amounts of substances being introduced to the global chemical market, and avoiding regrettable substitution caused by drop-in substitution. The idea of substance grouping based on the relationship between chemical structure and hazard has been applied in several cases already. Many of the very first hazardous organic substances to be regulated globally as pollutants were groups sharing a similar structure, including DDT-derivatives, dioxins, and PBDEs. This presentation will provide an overview of strategies for grouping hazardous substances based on chemical structure and how these substance grouping approaches may be applied in relation to persistent and mobile substances, as as these are a class of substances receiving increasing, global attention. This work will be particularly relevant to identify groups substances with either a common chemical moiety, or common transformation products, that are considered persistent, mobile and toxic (PMT) or very persistent and very mobile (vPvM) [1]. Since approaches are needed now to better assess and regulate them to protect drinking water sources, we explore how grouping persistent and mobiles substances based on specific molecular substructures will support PMT/vPvM substance prioritization for risk governance and assessments.

#### **3.04.D.T-04 Cigarette Butts as a Source of Organic Pollutants**

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Cigarette butts (CBs) are small residues with mixed composition. Produced in large amounts, their accumulation in the environment has become alarming. It is possible to classify more than 7000 chemical components generated either in the burning process or when distilled from the tobacco. The aim of our research is to describe the rate of release of phenolic compounds and polyaromatic hydrocarbons (PAHs) from CBs.

At first, attention was paid to the determination of phenolic compounds and PAHs in fresh CBs. Subsequently, the changes in the content of these compounds in CBs during exposure of the butts to external conditions were monitored. An analysis of the soil on which the cigarette butts were exposed was also carried out. Based on the measured data, it is possible to describe the fate of selected organic pollutants after discarding a cigarette butt into the environment. This research provides missing information on the phenolic content in CBs and the rate of their release into water. It also provides a description of the kinetics of the release of PAHs during exposure to cigarette butts. It thus complements previously published information on CBs as a source of environmental contamination.

### **3.04.P Analysis, Assessment and Management of Contaminants of Emerging Concern and Their Transformation Products in the Environment**

#### **3.04.P-Th150 Spatial and Seasonal Variations of Emerging Contaminants, Microplastics and Per- And Polyfluoroalkyl Substances (PFAS) in Pampulha Lake, a UNESCO Heritage Site in Brazil**

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The Pampulha Lake is part of a modern ensemble considered one of the main tourist attractions in Belo Horizonte, Brazil. The ensemble was included in UNESCO's World Heritage List in 2016. Despite its cultural and recreational importance to the local population, Pampulha Lake has gone through a process of deterioration of its water quality over time. Not only does the lake have no riparian forest, but it also receives domestic sewage and stormwater from drainage systems from neighborhoods within the watershed. As a result, the lake is vulnerable to anthropogenic pollution. Although recreation and fishing are prohibited, illegal fishing is common in the lake. This study provides the first broad assessment of the occurrence of emerging contaminants (EC) such as pesticides, pharmaceuticals, personal care products, hormones, microplastics (MP), and per- and poly-fluoroalkyl substances (PFAS) in the lake. Sampling was conducted under different weather conditions and throughout the lake to analyze potential seasonal and spatial patterns of occurrences. For organic compounds, sample preparation was carried out by solid-phase extraction (SPE), and a range of 29 analytes was quantified using LC-MS/MS. MP sample preparation used Fenton's reagent, followed by density separation using NaI ( $\rho = 1.6 \text{ g cm}^{-3}$ ). The final MP samples were analyzed by optical microscopy, and chemical characterization was made using near-infrared hyperspectral imaging (NIR-HIS) and micro-FTIR. MP were found at 28 – 450 items  $\text{m}^{-3}$  concentrations throughout the samples. The total sum of ECs varied between 250 and 1600  $\text{ng L}^{-1}$ , and PFAS were observed at levels between 34 and 70  $\mu\text{g L}^{-1}$ , an alarming environmental concentration. Seasonality affected the concentration of several ECs, such as atrazine, simazine, caffeine, and microplastics, but PFAS levels were unaffected. Spatial variations were also observed for the concentrations of many pollutants, indicating possible sources related to anthropic activities. Risk assessment indicates that concentrations observed in this study for 8 PFAS and 2 EC could threaten aquatic life. Our results suggest that a monitoring plan is needed to identify sources of pollution better and provide information for elaborating remediation strategies.

#### **3.04.P-Th151 Particles in the Aquatic Environment: Heteroagglomeration with Natural Suspended Particulate Matter**

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The determination of nanoparticles (NPs; manufactured nanomaterials, nanoplastics, natural colloids) transformation in the aquatic environment is a prerequisite for understanding nanoparticle fate and informed risk assessments. Environmental transformation comprises four main routes: dissolution/leaching, abiotic transformation/degradation, biotic transformation/degradation and (hetero-)agglomeration.

Heteroagglomeration kinetics are crucial for the understanding of NP transport processes. Parametrization of this process between NPs and suspended particulate matter (SPM) was hampered by the spatial and temporal variability of SPM floc composition and conformation/size. Analytical methods were either unsuitable or required unrealistic NP concentrations. The SPM used in most studies was either unrealistically simple (silica particles, clays) or rather unique (natural SPM).

After a thorough analysis of mechanisms of floc formation and the relevant building blocks of natural, riverine SPM and the reproducible laboratory synthesis of stable model SPM flocs, we designed a method to determine kinetics under environmentally relevant conditions, allowing well controlled laboratory experiments as well as standardization for risk assessment. The heteroagglomeration attachment efficiency ( $\alpha$ ) constitutes the most suitable parametrization of particle-particle interactions. The presented test matrix combines synthetic model SPM flocs with the model freshwater composition suggested in OECD TG 318, both designed to represent agglomeration-relevant characteristics of natural systems. The test matrix was employed in a newly developed stirred-batch method, addressing the shortcomings of existing strategies to determine ( $\alpha$ ). A highly sensitive analytical approach allowed to work at realistic concentrations of NP (5 ppb) and SPM flocs (20 and 40 ppm).

The test system was evaluated by testing the heteroagglomeration of CeO<sub>2</sub> nanoparticles in four different combinations of floc and water chemistry.

- Natural flocs in natural water
- Natural flocs in synthetic (TG318) water
- Synthetic flocs in natural water
- Synthetic flocs in synthetic water

The results show the applicability and precision of the invented test system and the synthetic flocs but also some differences between results from natural and synthetic water chemistry which can be explained by the type and quality of the NOM. Calculated transport distances for 50% unassociated NPs reached up to 370 km, what is unexpectedly high.

### 3.04.P-Th152 Occurrence and Distribution of Per- and Polyfluoroalkyl Substances in Serbian Surface Water

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The widespread use of per- and poly- fluoroalkyl substances (PFASs) in a numerous industrial and commercial applications causes contamination of different environmental compartments, representing *important risk factors*. The data dealing with the occurrence and distribution of PFASs in the Balkan Region including the Republic of Serbia are scarce and often limited regarding the number of analyzed compounds, so the intention of this work is to contribute to data on the chemical status of surface water with respect to this class of emerging pollutants. Thus, the aim of this study was to investigate the seasonal (summer vs. winter) and spatial distribution of selected PFASs in surface water samples of a few international rivers (Danube, Tisa, and Sava) and of an important irrigation canal system (Danube-Tisa-Danube), taken in the northern Serbian province of Vojvodina, a region known for high agricultural activity. Surface water samples were collected before and after the wastewater (WW) discharge point of each selected settlement, and in this way contribution of WW to the PFASs occurrence was also assessed. Sample preparation was based on the application of PFASs-specific solid-phase extraction sorbent followed by analysis on ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry. Selected surface water recipients differed among each other regarding the water flow bringing to different dilution of compounds presented in WW. Additionally, the size of nearby settlements and industrial activities are markedly different. The results will give insight into the occurrence and distribution (including seasonal variation) of PFASs in the main water systems of Serbia where absence of WW treatment plants has detrimental environmental effect, which cannot be estimated without revealing the levels and distribution of organic contaminants.

### 3.04.P-Th153 Combining Liquid Chromatography-High Resolution Mass Spectrometry and in Vivo Models to Assess Unexpected Thyroid-Active Transformation Products From the Degradation of Diclofenac

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Diclofenac (DCF) is an environmentally persistent, nonsteroidal anti-inflammatory drug (NSAID) with thyroid-disrupting properties. The extensive use of this endocrine-disrupting chemical (EDC) and its inadequate removal from wastewater result in its detection across all continents, including Antarctica. Efforts are being made to remove EDCs from wastewater using electrochemical advanced oxidation processes (eAOPs). However, these novel remediation technologies generate transformation products (TPs) with largely unknown chemical and biological characteristics.

Herein, to comprehensively assess the safety of eAOPs, DCF was electrochemically degraded using a boron-doped diamond anode. Liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) was used to perform suspect and non-targeted screening to analyze the TPs of DCF and elucidate its potential degradation pathways. To assess their biological impact, the thyroid axis activity of DCF and TPs was determined using the *Xenopus* Eleutheroembryo Thyroid Assay (XETA).

For the first time, this study revealed that exposure to DCF disrupts the thyroid hormone (TH) signaling in amphibians, by diminishing the activity of tadpoles' thyroid endocrine system. Unexpectedly, as DCF degradation progressed, the overall *in vivo* thyroid activity transitioned from an anti-thyroid effect in non-treated samples to a pro-thyroid effect in intermediately treated samples. This implies the emergence of thyroid-active TPs with distinct modes of action compared to the original compound. It is hypothesized that this pro-thyroid effect was caused by TPs generated through the oxidative cleavage of DCF's benzylic ring, giving rise to an aliphatic side chain with electronegative groups. Molecular docking analysis revealed that these TPs have strong binding affinity to the thyroid receptor, potentially triggering TH-like responses. Moreover, acute toxicity occurred in intermediately degraded samples, indicating the formation of TPs exhibiting higher toxicity than DCF. Nevertheless, both acute toxicity and thyroid-stimulating effects were mitigated with a prolonged degradation time.

In conclusion, the radical transformation of EDCs by eAOPs can produce TPs with unpredictable chemical and biological features, potentially posing risk to the environment and public health. This highlights the necessity of holistic and reliable chemical and biological tools for the environmental risk assessment of these remediation technologies.

### 3.04.P-Th154 Occurrence of organic pollutants and source apportionment in urban groundwaters

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The escalating exploitation of groundwater (GW) has raised awareness about the need for quality recognition and studies addressing the presence of contaminants in GW. Various pollutants, including pharmaceuticals, personal care products, artificial sweeteners, and industrial chemicals, may infiltrate in urban GW. The Directive 2006/118/EC amendment (2022) on groundwater quality and protection was issued [2]. While it covers persistent organic substances like carbamazepine and sulfamethoxazole, it does not yet encompass 24 other persistent compounds, including PFAS [1]. Our study focused on analyzing 122 pollutants covering a broad polarity range in urban groundwater, surface, sewer, rain water runoff (street & roof) samples from Barcelona using an evaporative enrichment methodology combined with LC-Orbitrap-MS to detect organic compounds including pharmaceuticals, industrial chemicals, and tire wear substances. Special attention was paid to the very mobile contaminants, whose analysis is particularly challenging. Multivariate statistical analysis was used to identify the origin and quantify the proportions of the different sources. In this study, groundwater, surface water, sewer, urban runoff (street and roof) water samples were collected in Barcelona metropolitan area. Two enrichment techniques were used: multisorbent solid phase extraction and vacuum-assisted evaporation. Prior to LC-MS analysis, eluates were evaporated and reconstituted in 0.5 mL of 5% or 95% of acetonitrile for analysis by C<sub>18</sub> or HILIC, respectively.

This study demonstrates the use of a combined analytical approach for the determination of a variety of organic compounds. Multivariate statistical analysis (Principal component analysis and Mixing Ratios Calculations model) allowed to capture differences between sources. The study highlighted a clear need for updating existing monitoring schemes. This will allow to monitor water quality in a more comprehensive way and to ultimately assess the threat of groundwater contamination with respect to its use as resource for drinking water

[1] Directive of the European Parliament and of the council, 2022. DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy.

### 3.04.P-Th155 Spatial and Temporal Analysis of Contaminants of Emerging Concern (CECs) in Dublin Bay: Presenting a Marine Biofilm as New Sampling Device

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Contaminants of Emerging Concern (CECs) are a mainly created by humans and they reach the marine environment mostly directly from land-based sources, but there are cases in which they are emitted or re-mobilized in the marine environment itself. CECs are a concern because of their high levels of human usage, and the biological effects exerted on nontarget organisms. This group of compounds is being considered in the European Union in the Marine Strategy Framework Directive Descriptor 8, together with the Water Framework Directive and the Regional Sea Conventions.

Therefore, the main objective of this study was to determine and quantify existing CECs present along the Dublin coast. This objective was threefold: (i) the use of biofilm as a sampling device, considering micro and macrofouling, using a lobster cage as support for the biofilm; (ii) the determination of CECs in 8 beach locations in Dublin Bay, including a UNESCO Biosphere; (iii) and the measurement of CECs in Poolbeg Marina over 5 days.

The biofilm study comprised a separate evaluation of micro and macrofouling which showed the same profile where the sunagents (avobenzone and octocrylene) presented the highest concentrations, particularly the avobenzone. Octocrylene showed significant differences between both fouling types, presenting a concentration of 0.15 ng/L in macrofouling and of 0.38 ng/L in microfouling. Thus, some compounds were equally quantified by both fouling types, but there are others that showed significant differences. The spatial analysis of CECs showed that four compounds represent the biggest proportion of the CECs determined. Malahide beach presented the highest concentration of these compounds, between 225 to 10,000 ng/L. On the contrary Dun Laoghaire and Howth Harbours presented the lowest concentration of these compounds because mainly boat activities predominate instead of swimming. The temporal analysis was carried in a marine location in close proximity to the outlet of the tertiary wastewater treatment plant. This location did not show any upward or downward trend in measured CECs during the sampling week. This is a conclusion in itself because it implies that there is a constant concentration of marine contaminants. With these results demonstrating a wide range of chemical type and location, it is necessary to consider the possible cumulative effect on the different species that can nest or inhabit the UNESCO biosphere – near each of the sampling sites.

### 3.04.P-Th156 Skimming the lake surface microlayer - how to catch highly lipophilic pollutants in surface water

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Switzerland (FHNW), Switzerland, (2)University of Applied Sciences and Arts Northwestern Switzerland (FHNW), Switzerland UV filters are used extensively in sunscreen products, but also in other cosmetic products and industrial goods to reduce the harmful effects of UV radiation. Apart from the direct exposure during swimming activities, UV filters are released into the aquatic environment in large quantities via sewage treatment plants. In this study we investigated the presence of UV filters in small, remote, prealpine and alpine mountain lakes. These lakes are not affected by UV filter inputs from sewage treatment plants, however recreational swimming activities in summer are thought to lead to inputs. Since most of the common UV filters are very lipophilic, we studied the concentrations of UV filters in the surface microlayer of the lakes instead of taking grab samples.

The fate of the UV filters was investigated in four different lakes, three of which are located in the canton of Graubünden and one in the canton of Zurich. The size of the lakes varied between 20'000 and 360'000 square metres. After sampling, the organic UV filters were preconcentrated by Oasis HLB solid phase extraction and afterwards determined on a high performance liquid chromatography coupled to triple quadrupole mass spectrometer.

The studied UV filters were ingredients currently used in sunscreens which were as follows 2-ethylhexylsalicylate (EHS), 2-phenylbenzimidazole-5-sulphonic acid (PMDSA), avobenzone (AB), ethylhexyl methoxycinnamate (EHMC), octocrylene (OC) and oxybenzone (OB).

The concentrations of UV filters reached values of up to 112 µg/L for OC, followed by 3.4 µg/L for AB and 2.4 µg/L for PMDSA. The UV filters EHS and EHMC were found in concentrations ranging from 0.02 to 1.2 µg/L whereas OB was in the range of 0.01 and 0.02 µg/L. The sampling of the surface microlayer showed very high values for OC, which has not been observed in surface waters so far based on previous literature. This is of particular concern as OC has a high potential for bioaccumulation which can lead to high concentrations in biota.

### **3.04.P-Th157 Assessing the risk of booster biocides for the marine environment: a case study at the Belgian Part of the North Sea**

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The biofouling of submerged surfaces such as ship hulls is often prevented by using copper-based anti-fouling paints containing booster biocides. These booster biocides enter the water column and may affect non-target organisms. So far, the environmental risks for booster biocides have barely been quantified in the North Sea. In this study, the concentration of five commonly used booster biocides as well as tributyltin has been measured at five dredged spoil disposal sites in the Belgian part of the North Sea and the harbour or ports of Nieuwpoort, Oostende, and Zeebrugge. In four out of five dredged spoil disposal sites, a concentration with a risk characterization ratio (RCR) of more than 1 was observed in 97% of the samples. This indicates that the current ambient booster biocide and tributyltin concentrations may pose a risk to the marine environment. Tributyltin, for example, has been banned since 2008, but was still detected with an RCR of 237 to 546. The contamination observed in a specific harbour or port corresponds to the respective dredged spoil disposal sites and can be linked to the type of traffic. Additionally, different hotspots were observed. For Irgarol, for example, a 55-fold higher concentration has been observed at one sample location in the Port of Oostende compared to the median concentration in that port.

High RCR values detected for booster biocides and tributyltin indicate the importance to monitor these components such that hotspots may be discovered, and that actions can be taken in time if the concentration of a component exceeds the threshold at which irreversible damage to the marine environment may be expected.

### **3.04.P-Th158 Wide-Range Target Screening of Pharmaceuticals in Serbian Rivers: Occurrence and Seasonal Distribution**

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In recent decades, the impact of anthropogenic activities on water resources, primarily on surface water, has grown significantly due to population growth, and expansion of urban, industrial, mining, and agricultural sectors in both developed and developing countries. Additionally, surface waters are increasingly contaminated because they also serve as recipients of municipal and industrial wastewater containing a wide variety of pollutants, which even after treatment end up in rivers and other water bodies. The number of contaminants of emerging concern that may be present in surface waters is enormous, and their fate and ecological impact are still largely unknown. Among numerous contaminants of emerging concern, pharmaceuticals cause global concern because they contribute to the development of antibiotic-resistant bacteria and threaten the long-term survival of many species. For broad surveillance of these pollutants, a target screening approach is used to help

clarify the chemical quality of surface waters. In light of the foregoing, the main objective of this study was to screen the presence of pharmaceuticals in several rivers in Serbia by using wide-range target analysis with liquid chromatography-tandem high-resolution mass spectrometry (LC-HRMS). This research is focused on the Danube, Tisza, Sava, Tamiš, and Begej rivers in order to characterize their chemical status taking into account that wastewater in Serbia has been mainly directly discharged without treatment into surface watercourses. Water samples were collected during two campaigns, the first organized during the winter and the second during the spring in order to obtain a more comprehensive spatial and seasonal distribution of the compounds of interest. Samples were prepared using housemade multilayer SPE cartridges, while the obtained extracts were analyzed by LC-HRMS. The results obtained in this study will bring new information about the quantified levels of the identified pharmaceuticals, about the most prevalent compounds in the studied Serbian rivers, and the spatial patterns in the detection of compounds at the sampling locations. Furthermore, the dataset on pharmaceutical contamination of surface watercourses in Serbia will be compared to the relevant data from other parts of Europe, creating a solid basis for future monitoring programs and expanding existing knowledge on the spread of these contaminants in the water resources of the Western Balkans.

#### **3.04.P-Th159 Analysis of Antiozonants, Vulcanizing Agents and Other Hazardous Organic Compounds in Tire Rubber Particles**

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During the tire manufacturing process, many chemical agents are added to the rubber, and many of them can be considered as environmental pollutants. Some of these hazardous organic compounds are the antiozonant 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine) or the vulcanizing agent DPG (1,3-diphenylguanidine). One way of recycling end-of-life tires consists of shredding them to obtain crumb rubber, the largest source of intentional microplastic pollution. This granulate material is used as infill in artificial turf sports fields and as playground flooring. The European Commission announced its ban on September (2023), allowing manufacturers 8 years to adapt to this new regulation. This prohibition shows the importance of alternative infill materials.

In this work, an analytical methodology for the determination of 11 hazardous organic compounds in tire rubber particles is proposed. The method is based on a simple extraction step using ultrasound energy (Ultrasound Assisted Extraction, UAE), followed by LC-MS/MS analysis. Small amounts of sample and solvent are required, as well as a short extraction time. The method was applied to a wide variety of real samples, including crumb rubber from football fields and playgrounds. In addition, several alternative materials were also analyzed for comparative purposes (sand, cork, and thermoplastic elastomers). The antiozonant 6PPD showed the highest concentration levels in the newly constructed fields, reaching values up to 0.2% w/w while DPG was detected in end-of-life tires at concentrations about 100 µg/g.

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#### **3.04.P-Th160 Accurate Microplastics Characterization on Aluminum-Coated Filter Using QCL IR Chemical Imaging**

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Indeed, microplastic pollution has become a significant environmental concern due to its potential impacts on ecosystems and human health. QCL Infrared Chemical Imaging System represent an innovative approach to imaging and spectral analysis, particularly in the context of microplastic identification and other applications that require chemical characterization of materials.

Depending on the level of suspended solids presents, ASTM D8333 describes the steps required for microplastics sample preparation. Regardless of analysis technique used, samples with various levels of microplastics eventually require a filtration step. At present, for QCL IR Imaging systems microplastics sample introduction can be achieved directly on gold-coated filters.

To minimize the cost associated with gold-coated filters while maintaining easy and efficient sample introduction method, accurate microplastics characterization with IR QCL can be performed on aluminum-coated polyester filters. This study outlines the utilization of QCL IR Imaging in achieving accurate microplastics characterization on aluminum-coated filters in terms of particle detection, particle count repeatability, particle size accuracy and identification of common microplastics.

#### **3.04.P-Th161 Assessment of Volatile Organic Compounds (VOC) in underground water from around fuel station in Okinni Oshogbo, Osun State, Nigeria**

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Levels and possible toxicity of volatile organic compounds (VOC) were investigated from underground water in six locations around the vicinity of the fuel station within the Okinni locality, Osun State, Nigeria. Samples were collected and analyzed following the standard procedure. A total number of seven VOCs were detected with varying concentrations, they were: benzene, toluene, chlorobenzene, ethylbenzene, m, p-xylene, o-xylene, and dichloromethane (DCM). The concentration of benzene in this study was 0.241 mg/L which was higher than the Maximum Contaminant Level of 0.005 mg/L recommended by the United States Environmental Protection Agency (USEPA). Also, higher than the 0.01 mg/L permissible limit in drinking water as provided by the World Health Organization (WHO). Toluene ranges from 0.1125 mg/L to 67.194 mg/L which was greater than the threshold value of 1.0 mg/L in drinking water by USEPA in all locations except for locations L4, (where it was not detected) L5 and L6, and were higher than 0.7 mg/L permissible concentration limit in drinking water as recommended by WHO. The observed values of chlorobenzene range from 3.323 to 17.976 mg/L and were in multiple fold higher than the permissible value of 0.1 mg/l by USEPA. It was observed that the concentration of ethylbenzene in the sampling sites was far higher than 0.7 mg/L recommended by USEPA and the 0.3 mg/L threshold value permissible by WHO except for sites L3 which has values of 0.632 mg/L in this study, the concentration of isomers of xylene observed ranges from 0.124 mg/L to 18.427 mg/L and 0.735 to 22.105 mg/L for m, p-xylene and o-xylene respectively. It was higher than 5.0 mg/L and 10.0 mg/L permissible value in water by WHO and USEPA respectively in all the studied sites except for locations L4-L6. The concentration of DCM ranges from 0.644 mg/L to 6.253 mg/L. It was higher than the threshold value of 0.005 mg/l recommended by USEPA in all the studied sites. Most of the detected VOCs belong to the gasoline hydrocarbon VOC group. This suggested that they were mostly leachate from the fuel station. Thus, using the water samples collected from these locations for domestic activities could expose the people of these communities to serious health issues. Therefore, there is a need for urgent attention from the relevant authorities to safeguard the health of this populace.

#### **3.04.P-Th162 Degradation of phenol and sulfamethoxazole with persulfate and ozone with nano-MnO<sub>2</sub>-biochar composites**

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In this study, nano-MnO<sub>2</sub>-biochar composites were synthesized as catalysts to promote the oxidation of recalcitrant organic contaminants. The MnO<sub>2</sub> nanoparticles coated on biochar were hypothesized to enhance the oxidation of phenol and sulfamethoxazole (SMX) using ozone and/or persulfate. The optimal percentage of MnO<sub>2</sub> coating on the surface of biochar was determined, and the synthesis of nano-MnO<sub>2</sub>-coated biochar was confirmed via scanning electron microscopy-energy dispersive X-ray spectroscopy (EDS), X-ray diffraction, and EDS line mapping analyses. Compared with oxidation using ozone or persulfate in the presence of either nano-MnO<sub>2</sub> or biochar, the synthesized nano-MnO<sub>2</sub>-biochar composite markedly enhanced the oxidation of phenol and SMX. This was probably due to the spreading of MnO<sub>2</sub> nanorods on the surface of biochar and the synergistic involvement of nano-MnO<sub>2</sub> and biochar as catalysts. X-ray photoelectron spectroscopy analysis confirmed the involvement of Mn<sup>3+</sup> in MnO<sub>2</sub> to promote oxidation using ozone or persulfate. The co-existence of ozone and persulfate could also promote oxidation. Hydroxyl radicals (OH<sup>•</sup>) and persulfate radicals (SO<sub>4</sub><sup>•-</sup>) were major reactive oxygen species for ozone and persulfate systems, respectively. Results suggest that the synthesized nano-MnO<sub>2</sub>-biochar composites may be effective catalysts for various types of advanced oxidation processes in water and wastewater treatment.

#### **3.04.P-Th163 Biotransformation of Metronidazole, Sulfamethoxazole, and Trimethoprim by The Green Microalga *Chlorella sorokiniana*: Removal Efficiency, Mechanism, and Pathways**

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Assessing the efficiency of wastewater treatment in eliminating pharmaceutical contaminants from water is important and also reduces the effect on the environment when effluent is released. It is crucial not only to monitor the percentage of total removal of pharmaceutical pollutants but also to examine the transformation products (TPs) that formed during the process. TPs are normally more inconstant than their parent molecules and may also be more durable and more toxic.

We have used, *C. sorokiniana* to assess the biotransformation of three antibiotics (metronidazole: MTZ, sulfamethoxazole: SMX, and trimethoprim: TMP) both individually and in a mixture. In the single antibiotic experiments, removal efficiency for each antibiotic was higher when compared to mixed antibiotic cultures. The antibiotic removal mechanisms involved were biodegradation > photodegradation > bioadsorption > bioaccumulation. More efficient antibiotic removal was observed for MTZ > TMP > SMX > mixed antibiotics. Higher algal growth at the end of the experiment was shown in the presence of MTZ > mixed antibiotics > SMX > TMP.

The analysis of the transformation products from the supernatant samples of individual antibiotic experiments was carried out by ultra-high-performance liquid chromatography coupled with mass spectrometry. The degradation of MTZ, TMP, and SMX resulted in six, four, and ten TPs, respectively. Based on these observations TPs, three transformation pathways are suggested, with total transformation products in the MTZ, TMP, and SMX pathways showing 18, 19, and 41 TPs, respectively.

These findings suggest that *C. sorokiniana* represent an interesting microalga for antibiotic removal in wastewater.



*Keywords: Chlorella sorokiniana, Microalga, Transformation products, Transformation pathways, Antibiotics*

### **3.04.P-Th164 Occurrence of Illicit Drug Residues and New Psychoactive Substances in Wastewater Influent in the Region of Sfax, Middle East of Tunisia.**

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Numerous studies confirm the contamination of wastewater with organic compounds, including residues of drug of abuse (DAs) and novel psychoactive substances (NPS) at minute concentrations. This research investigated the presence of these emerging micropollutants in influent wastewaters (IWW) from five Tunisian Wastewater Treatment Plants (WWTPs). Composite samples of influent wastewater (collected over 24 hours) were gathered continuously for 7 days in November 2021. A well-optimized multi-residue liquid chromatography tandem mass spectrometry (LC–MS/MS) method was employed to identify and quantify 11 drug of abuse compounds or their metabolites. Among the sewage plants studied, cannabis and ecstasy (MDMA) were the most frequently detected substances. In this study, a novel wastewater-based epidemiology (WBE) approach was introduced to estimate illicit drug usage, enabling the calculation and assessment of collective illicit drug consumption at a community level. This approach relied on the concentrations of specific illicit substances and their primary metabolites in influent wastewater. The average cannabis consumption in the examined cities ranged from 1.6 to 31.93 g/day/1000 inhabitants and exhibited an increase on weekends. MDMA consumption ranged from 0.1 to 7.26 g/day/1000 inhabitants. Additionally, a qualitative investigation of new psychoactive substances (NPS) was conducted for the first time in an African nation, evaluating the presence of 33 NPS in wastewater samples. Out of these 33 NPS screened across all sampling sites, 17 were provisionally identified through this methodology. These 17 detected NPS encompassed a wide range of representative molecules from various NPS categories, including synthetic opioids, synthetic cathinones, derivatives of amphetamines, and synthetic cannabinoids. The highest detection frequency was recorded in the weekend particularly for synthetic cathinones and amphetamines derivatives (alpha-PHPP, mephedrone, pentedrone, pentylone, ethylone, MDA, MDEA), although synthetic cannabinoids (JWH-250, JWH-073, CP47497, HU-210) were much higher across the sampling week than other detected NPS in this study. This seems consistent that mainly stimulant drugs have been found, as music festivals and nightlife venues are more prone to the use of this category of NPS during weekend.

### **3.04.P-Th165 Rapid Multi-analyte Quantification of Disrupting Contaminants in Surface Water Through the Use of Deep Eutectic Solvents and Tandem Mass Spectrometry**

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There is a wide range of anthropogenic contaminants targeted in water resources. Among these are the "disrupting contaminants" including pharmaceutical and personal care products, bisphenols, and pesticides, used in a growing scale. These contaminants pose environmental challenges due to their persistence and the ability to cause specific physiological effects even at low concentrations. Despite wastewater effluents represent just one potential source, they are considered the primary pathway for these compounds to enter the aquatic environment, mainly because most wastewater treatment plants are not prepared to eliminate them. The impact on the quality of river water is crucial for environmental risk assessment and often leads to unpredictable effects on wildlife and human health. The aim of the present work is the development of a rapid and environmental friendly analytical procedure for determination of 31 disrupting contaminants in surface waters. Several personal care products, namely fragrances and UV-filters, bisphenols, and pesticides were subject to dispersive liquid-liquid microextraction (DLLME) using a deep eutectic solvent (DES) as extractive solvent. Gas chromatography - tandem mass spectrometry analysis was performed with electroionization by using multi reaction monitoring (MRM). During method development, a comparison of different types of DES, extractive and dispersive volumes, and extraction times was performed using a chemometric approach. The developed method was validated according to European guidelines criteria by determining linearity, precision, recovery, limits of quantification and limits of detection.

### **3.04.P-Th166 Antimicrobial exposure with Eisenia fetida earthworms: multitarget analysis, by-products identification and biological effects elucidation**

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The overuse of antimicrobials (AMs) has led to their presence in different untargeted environmental matrices, which is of global concern due to the emergence of AM-resistant strains. Earthworms are sensitive to contaminants in soil, therefore, they are considered adequate model organisms in terrestrial ecotoxicology to evaluate the introduction of contaminants, such as AMs, into the food chain. The determination of AMs in earthworms is still a field to be further investigated since the complex biological matrix together with the different physicochemical properties of the different AM families make the simultaneous analysis difficult. In addition, little is known about the degradation of AMs in the organism and their effects. In that context, in

this work an analytical method has been validated, which showed adequate accuracy (apparent recoveries in the range of 80-120 % and procedural limits of quantification of 1.4  $\mu\text{g}\cdot\text{kg}^{-1}$  - 9.6  $\mu\text{g}\cdot\text{kg}^{-1}$ ) for the simultaneous determination of ten AM families in earthworms. Moreover, OECD-207 toxicity test was performed, exposing *Eisenia fetida* organisms to soils spiked with 10  $\text{mg}\cdot\text{kg}^{-1}$ , 100  $\text{mg}\cdot\text{kg}^{-1}$  or 1000  $\text{mg}\cdot\text{kg}^{-1}$  of sulfamethazine (SMZ) and tetracycline (TC), individually. The results confirmed the bioaccumulation of both AMs in the organisms, showing a greater tendency for the accumulation of SMZ since higher bioconcentration factor values were obtained for this compound at the different exposure concentrations tested. Moreover, degradation of both AMs has been detected in both matrices; successfully identifying thirteen transformation products (TPs) in soil and eight of them in earthworms too and their trend over time have been studied. Regarding the biological effects, only SMZ caused changes in earthworm growth, evidenced by weight loss in earthworms exposed to concentrations of 100  $\text{mg}\cdot\text{kg}^{-1}$  and 1000  $\text{mg}\cdot\text{kg}^{-1}$ . The riboflavin content decreased in all concentrations of SMZ, as well as in the highest concentration of TC, suggesting that these AMs have the potential to disturb the immune system of *Eisenia fetida*. This work supposed a step forward in broadening the understanding of AM contamination and the effects at the lower end of the food chain, both chemically and biologically.

#### **3.04.P-Th167 A Comprehensive Pollutant Profile Characterization of Sewage Sludge: The effect of different polarity fractions on compound recovery and toxicological profile using Accelerated Solvent Extraction**

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Sewage sludge is commonly applied as fertilizer on agricultural fields due to its favorable levels of phosphorus and organic matter, thereby increasing soil fertility and contributing to closing nutrient loops. However, the presence of a diverse range of emerging micropollutants has raised concern around this practice. While optimized extraction and chemical analysis methods exist for individual pollutant groups, there is a pressing need for a more comprehensive method.

This study aims to fill this gap by adapting and combining an Accelerated Solvent Extraction (ASE) with different sludge pre-treatments, resulting in different polarity fractions. The aim is to evaluate the impact on the extraction and recoveries of target compounds as well as the toxicological profiles.

Using a spike-addition approach, extraction efficiencies of apolar contaminant groups such as polychlorinated biphenyls, dioxins and furans, polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, and siloxanes were tested. Polar contaminant groups, like the 22 per- and polyfluoroalkyl substances (PFAS) specified in Denmark's recent sludge directive, and a mix representing widely used pharmaceutical classes, were also included. Sewage sludge samples were obtained from an urban wastewater treatment plant (Uppsala, Sweden).

Extractions were conducted with two different solvents (*n*-hexane and methanol) as well as different pre-treatments of the sludge (acidic digestion, alkaline digestion, no digestion) prior. Chemical analysis was performed with GC and LC-HRMS instruments for the hexane and methanol extracts respectively. Additionally, unspiked extracts were analyzed using an aryl hydrocarbon receptor (AhR) responsive DR CALUX® bioassay.

Recoveries of spiked compounds in the apolar extracts differed with pre-treatments and for this fraction, highest recoveries were achieved with no prior digestion. The bio-TEQ values derived from the DR CALUX® assay differed with pre-treatment and were also highest for the undigested fraction, reflecting different extraction efficiencies of AhR-activating compounds.

ASE combined with HRMS-GC and LC analysis seems to be promising to extract and characterize a wide range of pollutants present in sewage sludge and different pre-treatments should be considered as the resulting polarity affects extraction efficiency of pollutants to be monitored. Bioassays like DR CALUX® can give further insights into extraction efficiencies of bioactive compound classes.

#### **3.04.P-Th168 Investigation of pesticides, pharmaceuticals and personal care products in small water bodies using polar organic chemical integrative samplers (POCIS) and non-target screening**

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Pharmaceuticals and personal care products (PPCPs) as well as pesticides are classes of organic pollutants that pose a growing concern in the context of circular economy strategies due to their potential adverse impacts on both biota and human health. This study focuses on the occurrence of PPCPs and pesticides in three small water bodies located in Norway. To assess the presence of these contaminants over an extended period, polar organic chemical integrative samplers (POCIS) were employed as sampling devices.

Over the course of one year, water samples were collected at three-week intervals and subsequently analyzed using non-target screening on HRAM Orbitrap LC-MS. Confirmation of identified compounds was achieved through the utilization of reference standards. The aim of this study was to comprehensively investigate the distribution and concentration levels of PPCPs and

pesticides in the selected water bodies, shedding light on their potential impact on the surrounding ecosystem and human health.

The results of this study will contribute to a better understanding of the prevalence and persistence of PPCPs and pesticides in aquatic environments. Additionally, the utilization of POCIS in combination with advanced analytical techniques offers a valuable approach for comprehensive and time-resolved monitoring of these contaminants. The findings underscore the importance of implementing effective strategies to mitigate the release and accumulation of PPCPs and pesticides in water bodies, thereby promoting sustainable practices within the circular economy framework.

### **3.04.P-Th169 Assessing the Ecotoxicity of Thymol and Carvacrol Isomers on Aquatic and Terrestrial Organisms**

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Thymol and carvacrol, natural monoterpenic isomeric phenols, possess diverse bioactivities, notably as potent antimicrobial agents against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella thymimurium*, and *Pseudomonas aeruginosa*. Despite their benefits, their environmental impact on aquatic and terrestrial organisms remains poorly understood, necessitating a comprehensive assessment for environmental toxicity

This study seeks to compare the ecotoxicological effects of thymol and carvacrol on aquatic and terrestrial organisms, employing *Daphnia magna*, *Vibrio fischeri*, *Allium cepa*, and *Eisenia fetida* as test subjects.

*D.magna* and *V. fischeri* served as biological indicators for aquatic environments. The acute toxicity of thymol and carvacrol on *D. magna* was examined with concentrations of 2, 4, 6, 8, and 12 mg/L. Mobility effects were assessed 24 hours post-exposure, yielding Thymol EC<sub>50</sub>(24h) = 8.131 (7.298–9.203) mg/L, EC<sub>10</sub> (24h) = 12.494 (10.655–17.113) mg/L, Carvacrol EC<sub>50</sub>(24h) = 4.751 (3.936–5.523) mg/L, and EC<sub>10</sub>(24h) = 9.529 (7.802–13.755) mg/L. The dose-response curves for both compounds exhibited similar shapes, indicating a steep dose response, with carvacrol proving more toxic. *V. fischeri* fluorescence inhibition results after 30 minutes revealed thymol EC<sub>50</sub> = 6.20 (5.150-6.945) mg/L and carvacrol EC<sub>50</sub> = 0.585 (0.487 -0.701) mg/L.

*A. cepa* and *E. fetida* were used as indicators for terrestrial environments. *A. cepa* exposed to concentrations of 0.03, 0.3, 3, 30, and 300 mg/L of both compounds exhibited root inhibition impact after 72 hours, resulting in phytotoxicity EC<sub>50</sub> values of 4.048 mg/L (3.449–4.756 mg/L) for thymol and 6.466 mg/L (5.679–7.370 mg/L) for carvacrol. Thymol demonstrated greater toxicity at low to medium doses, while carvacrol inhibited root growth more significantly at high doses. *E. fetida*, after 14 days of exposure, showed high toxicity, with an EC<sub>50</sub> of 7.520 (6.097 - 9.109) mg/Kg for thymol and 0.967 (0.818 - 1.565) mg/Kg for carvacrol. Carvacrol exhibited greater toxicity against both aquatic organisms and *E. fetida*, while for *A. cepa*, thymol presented greater toxicity.

This study underscores the potential toxicity of thymol and carvacrol on environmental organisms. Despite their promise as alternatives to aggressive antimicrobial products, careful consideration of their environmental impacts is crucial.

### **3.04.P-Th170 Contamination of agricultural soils by micropollutants in irrigated areas of the Czech Republic**

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Agricultural soil can be contaminated by various micropollutants. One of the sources of these contaminants is agricultural practice, where a variety of pesticides are used for plant protection. The spectrum of the pesticide depends on the cultivated crops. Another source of contamination can be river water that is often used for irrigation. This water may contain various micropollutant including pesticides, pharmaceuticals, and personal care products. River contamination depends on the sources of the compounds, that can be, for example, discharged wastewater, surface water runoff from agricultural or urban land, etc. Therefore, contamination can vary both between individual rivers and along one river. Our study focused on soil contamination in areas irrigated with water from three rivers in the Czech Republic. These were mainly plots on which vegetables were grown (lettuce, onions, celery, parsley, carrots, beets, or potatoes), but also plots on which corn or buckwheat was grown. Targeted LC-MS<sup>2</sup> methods were used to measure concentrations of selected pesticides, pharmaceuticals, and their metabolites. In total 73 pesticides and their metabolites were found in the entire set of soil samples. The concentrations of 10 pesticides and their metabolites were > 20 ng/g of dry soils. The number of pesticides and their metabolites quantified in one soil sample varied between 28 and 52. The maximal sum of their concentrations in one soil sample was around 1500 ng/g of dry soil. The number of pharmaceuticals and their metabolites quantified in one soil sample varied between 1 and 7. The maximal sum of their concentrations in one soil sample was around 6 ng/g of dry soil. In addition, nontargeted LC-HRMS full scan/DIA methods were deployed to identify other pollutants in soils. By this approach, 49 additional pesticide related compounds were identified at Schymanski level 2. Semiquantitative comparison of peak areas revealed the gap of seven highly abundant compounds

between the first and second quantified pesticide. This fact documents the importance of a nontargeted approach for local monitoring setting and pesticide management. Principal component analysis did not reveal the dependence of pesticide occurrence on sampling site, i.e., on crop or source of irrigation water. The work was supported by the Ministry of Agriculture of the Czech Republic, project No. QK23020018.

### **3.04.P-Th171 Groundwater vulnerability maps of the Czech Republic specified for selected micropollutants**

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Soil can be contaminated by various micropollutants if treated wastewater or surface water that has been contaminated by this source are used for irrigation. Another source of contamination can be sewage sludge from wastewater treatment plants that is frequently used for soil enrichment. These contaminants can be taken up by plants or migrate through the soil environment and subsequently contaminate groundwater. Their leaching from soils and migration towards groundwater depends on the climatic conditions, properties of the vadose zone environment and behavior of a particular compound, i.e., its sorption onto soils and sediments, and stability in the environment. The Freundlich sorption isotherms were evaluated for twenty-one micropollutants (mainly pharmaceuticals) and representative soils of the Czech Republic. Multiple linear regressions were used to derive equations for predicting the Freundlich sorption coefficient ( $K_F$ ) using the properties of tested soils. These equations, the soil map, and the database of soil properties were used to predict the  $K_F$  value distributions within the Czech agricultural soils and subsequently to delineate classes of compounds' mobility in the soil environment (i.e., mobility index). The dissipation and half-lives of all micropollutants were also evaluated for the representative Czech soils. This information was used to define compound's stability index. General groundwater vulnerability map (i.e., distribution of the DRASTIC vulnerability index) was derived using the DRASTIC method based on the climatic conditions and properties of the vadose zone. Next, specific groundwater vulnerability maps for each compound were obtained by combining the DRASTIC vulnerability index, mobility index and stability index. The resulting maps of specific groundwater vulnerability were confronted with the results of groundwater monitoring that is carried out by the Czech Hydrometeorological Institute. This information will be used to improve the monitoring of groundwater quality within the Czech Republic. The work was supported by the Ministry of Agriculture of the Czech Republic, project No. QK23020018 and QK21020080.

### **3.04.P-Th172 Optimisation of SPE and LC-MS for the Determination of Antibiotic Concentrations in Liquid Environmental Samples**

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In 2015, an estimated 11,134.97 tonnes of antibiotics were consumed by humans in 59 countries. This figure is projected to increase to 128 billion doses by 2030. Antibiotics that are not fully metabolised by the organism end up in wastewater treatment plants. Conventional wastewater treatment plants cannot completely remove antibiotics, which remain in anaerobic treated effluents (ATEs). ATEs themselves lead to the occurrence of antibiotic resistance genes due to their interaction with microbes in treatment facilities. This establishes a clear link between antibiotic use and antibiotic resistance, which causes over 700,000 deaths per year. Thus, it is necessary to accurately measure antibiotic levels in the environment, but the precision of existing methods is limited by the occurrence of matrix effects. Matrix effects are an increase (ion enhancement) or decrease (ion suppression) in the response of the target analyte. Due to this, matrix effects can dramatically alter the performance of a method.

The aim of this work was to determine and measure the presence and concentration of antibiotics in water using Solid Phase Liquid Extraction (SPE) and Liquid Chromatography-Mass Spectrometry (LC-MS). SPE was performed using Oasis HLB 200  $\mu\text{g}$  cartridges, and the retained analyte was eluted with acetonitrile and reconstituted in a 10:10:80 methanol:acetonitrile:water solution. LC-MS/MS analysis was completed using an Agilent HPLC instrument with a 1290 Infinity II LC multi-sampler and temperature-regulated sample tray and column compartment. The columns used were a Zorbax eclipse plus C18 2.1 x 50 mm 1.8  $\mu\text{m}$  LC column and a Zorbax eclipse plus C18, 2.1 x 5 mm, 1.8  $\mu\text{m}$  UHPLC guard column at 30 °C. A 6470A triple-quadrupole mass spectrometer with electrospray ionisation (Agilent Technologies) was used for detection. Helium was used as a collision gas and  $\text{N}_2$  as a nebulising and desolvation gas. Data was collected using MassHunter software. The antibiotic sulfamethoxazole (SMX) was detected in each of the investigated surface water bodies in Ireland. The highest detected concentration was 3.2 ng/L showing a matrix effect of 321% (6.33% matrix effect SD).

At present, there is no method developed for the detection of antibiotics that is not subjected to inaccuracies due to matrix effects. The developed optimised method allows for the precise and accurate detection of antibiotics in water samples, by decreasing the occurrence of matrix effects to improve sensitivity.

### **3.04.P-Th174 Identification of Chlorinated Paraffin Biotransformation Products in Sediment Cores of a Polluted Lake: Insights into Oxidative Degradation**

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Due to their inherent persistence and bioaccumulative potential, the widespread application of organohalogenes in our society poses threats to public health and ecosystems. However, our capacity is inadequate to assess these risks, considering innumerable unknown organohalogenes, including undocumented chemicals, byproducts, and transformation products.

Therefore, disclosing unknown organohalogenes is an everlasting challenge for environmental analytical chemists. Leveraging relatively smaller molecular sizes of organohalogenes, ion mobility spectrometry-derived collision cross section (CCS) is a promising dimension to flag unknown organohalogenes in massive nontarget data. Herein, ion mobility-high-resolution mass spectrometry coupled to high performance liquid chromatography was used to depict depth profiles of substances in sediment cores from Lake Tyrifjorden, Norway. This lake has been reported to be heavily polluted by emissions from a factory producing paper products. After peakpicking, alignment, and cleanup, 218 features were prioritized as potential emerging organohalogenes with smaller sizes ( $CCS < 100 + 0.2 \times \text{mass}$ ) and significantly increasing trends with the year of deposition ( $p < 0.05$ ). Among those features, 113 showed a typical chlorine isotopic distribution, comprising 44% of the total intensity of potential emerging organohalogenes in the uppermost layer of the cores, suggesting a large volume of use in the catchment. Based on isotopic patterns, exact masses, and fragment ions, element compositions of 34 unknown compounds were derived and grouped as potential oxidized chlorinated paraffins ( $C_{24-26}H_{37-43}Cl_{9-11}O_3$ ) and sulfated chlorinated paraffins ( $C_{24-26}H_{36-47}Cl_{7-12}SO_5$ ,  $C_{24-26}H_{36-43}Cl_{9-11}SO_6$ , and  $C_{26}H_{43}Cl_9SO_7$ ). To validate our deduction and further investigate their formation mechanism, biodegradation experiments were conducted using clean upstream sediments. Functions of CCS vs. mass were established to predict CCS of all potential chlorinated paraffin biotransformation products. *In situ* increasing ratios of oxidized products over potential precursors with the depth indicated that even in the deeper layer of sediment (~10 cm), oxidative degradation still prevailed over reductive dechlorination. This study reports a CCS-oriented method to readily acquire profiles of chlorinated paraffin biotransformation products, and empirical evidence of the dominance of oxidative elimination of chlorinated paraffins in typical lake sediments.

### **3.04.P-Th175 Analytical Method Validation, Residue and Health Risk Assessment for Clopidol in Egg Using Liquid Chromatography-Tandem Mass Spectrometry**

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Clopidol is extensively used in livestock farming and residues of this antibiotic can persist in animal tissues, posing a risk to humans and the environment. The analytical method was validated with respect to parameters such as specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, and precision. Additionally, the accuracy and precision were assessed based on three replicate samples at four different spiking concentrations. As a result, no interference peak was observed to affect the quantification of clopidol in the pretreatment methods, and the LOD and LOQ values were 0.0005 and 0.001 µg/g, respectively. The concentrations of calibration standards in the method ranged from 0.1 to 20 ng/mL ( $r > 0.99$ ). The accuracy of the pretreatment method was assessed through recovery results, and precision was estimated using the coefficient of variation (CV). The mean accuracies ranged from 71.74% to 89.85%, while the CV ranged from 0.2% to 7.1%, respectively. Consequently, these results are within the acceptable ranges specified in the guidelines for analytical method validation. In this study, following 14 days of exposure (after administering a clopidol-contaminated diet), clopidol concentrations were highest in eggs (median: 9.83 mg/kg) compared to other tissues (liver, fat, kidney, and muscle). A cumulative risk assessment for clopidol in eggs using the hazard quotient (HQ) method was conducted. The HQ values exceeded 1 for all age groups, especially for young children (<18 years).

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### **3.04.P-Th176 Analytical Method Validation and Residue Kinetic of Fumagillin in Rainbow Trout Using Radiolabeled Compound**

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Fumagillin is a compound isolated from the fungus *Aspergillus fumigatus* and is widely employed as an antimicrobial agent for honeybees and fish. It shows promise as both an anti-infective and antiangiogenic agent. In this study, we investigated the magnitude, dissipation, and metabolism of fumagillin, radiolabeled as [3H]fumagillin, in rainbow trout (fillet). Prior to analyzing the tissues collected from the test animals, specificity, linearity, system suitability, and recovery (accuracy and precision) tests were conducted. All results are within the acceptable ranges specified in the guidelines for analytical method validation. Fumagillin was administered orally, and the average dose administered, based on the body weight of the rainbow trout, was approximately 50.1 mg/kg after acclimation for Day 7. Ten rainbow trout were sacrificed at 6, 12 hours, Day 1, 2, and 7 after dosing, and fillet tissues were collected. The total radioactivity residue (TRR) in the extract of each tissue was

determined by LSC analysis, followed by Radio-HPLC to separate fumagillin and its metabolite. Radio-HPLC analysis showed that no metabolites other than the parent compound were detected in the fillet tissue extract. The concentration of fumagillin increased from 1.2 mg/kg to 2.3 mg/kg at 12 hours following dosing and then declined to 0.2 mg/kg on Day 7. The unextractable residues remained within the range of 1.2 to 1.7 mg/kg but significantly declined to 0.4 mg/kg by Day 7.

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### **3.04.P-Th177 Analysis and Occurrence of UV Filters, Parabens and Benzotriazoles in Cadiz Bay (Andalusia, SW Spain)**

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The present study provides new insights into the environmental distribution of two families of personal care products (PCPs) in estuarine systems in Cadiz Bay (southwest Spain): ultraviolet radiation filters (UV-Fs) widely used in sunscreens, and parabens (PBs) which are mainly used as preservatives in cosmetic products. Additionally, benzotriazoles, a group of high production volume compounds (HPVCs) that are used daily in various applications, were studied in this work. Despite being present in relatively low concentrations (parts per billion, ppb to parts per trillion, ppt) in the aquatic environment, these selected compounds of emerging concern (CECs) are capable of generating imbalances in the environment and producing toxic effects in organisms.

For this purpose, first, ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) analysis method was optimised to determine the selected PCPs. Then, solid phase extraction (SPE) and pressurised liquid extraction (PLE) techniques were optimised for the extraction of these compounds in liquid and solid samples, respectively. Environmental samples from both wastewater and aquatic systems of the Cadiz Bay (Guadalete River Estuary and San Pedro River Tidal Creek) were analysed to determine the presence and distribution of PCPs using the previously optimised analytical method. To assess the environmental risk in the study area, hazard quotients (HQs) were evaluated.

The multi-residue analytical method developed obtained acceptable results for 15 PCPs, of which 15 were detected in aqueous samples and 9 in solid samples.

The wastewater analysis provided removal rates of these compounds, which were higher for UV-Fs, parabens and 1H-Benzotriazole (BTA) when considering not only the dissolved fraction, but also the particulate fraction in the wastewater samples (2.84 % for UV-Fs, 34.10 % for parabens and 48.11 % for 1H-Benzotriazole). Also, in the Guadalete River Estuary a higher incidence of UV-Fs and PBs in summer was observed. Selected compounds such as BTA showed conservative behaviour along the Estuary, serving as an indicator for sewage contamination. Concentrations of UV-Fs and PBs in the sediment of the Guadalete River (202.78 and 37.3 ng·g<sup>-1</sup> respectively), were higher than those registered in the sediment of the San Pedro River Tidal Creek (78.66 and 13.49 ng·g<sup>-1</sup>).

Finally, BTA and EthylPABA were the two compounds with the highest risk coefficients (HQ higher than 1).

### **3.04.P-Th178 Degradation of the Herbicide Profoxydim by Ozone Water Treatment. Theoretical DFT Study to Elucidate the Reaction Mechanism**

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Drinking water sources are subjected to disinfection processes in order to achieve an adequate control of pathogenic microorganisms in the final drinking water. In this sense, ozone is one of the most innovative treatments in drinking water treatments due to its oxidizing and bacteriological power against waste. Ozone is also capable of reacting with organic pollutants such as pesticides present in water. Profoxydim is an herbicide used on rice crops that can reach natural water reservoirs intended for human consumption or reuse for agricultural purposes. Therefore, the objectives of this work were to study the behavior of this herbicide in the presence of ozone and to identify the possible degradation products (DPs). Furthermore, a theoretical study will be carried out to identify the main reaction mechanism, in order to explain the data obtained experimentally.

The kinetic evolution of aqueous solutions of profoxydim in the presence of ozone generated by a lab ozonizer was followed by HPLC-DAD. The assays were carried out at different ratios of Profoxydim: ozone (1:1/1:5/1:10). The identification of the main DP formed was performed by HPLC-ESI-QToF. The results showed that the herbicide was fast degraded by ozone under the conditions studied. A main and more stable DP was detected which tentatively identified as a by-product resulting from the oxidation of the sulfide moiety of the herbicide, leading to the corresponding sulfoxide. This byproduct has also been detected in other water disinfection treatments studied, such as chlorination.

The reaction mechanism for the formation of these oxidation by-products is not clear and there is no consensus in the literature. Therefore, a theoretical-computational study was carried out by means of DFT calculations using the basis set (B3LYP/6-311G+ (d, p)) to elucidate which oxidation pathway of Profoxydim sulfide (ionic or radical) is the most likely. The results may be useful to know how other pesticides might evolve under these conditions.

### **3.04.P-Th179 Migration of Organic Compounds from Rubber Materials in Contact with Water and their Safety Evaluation as Water Contact Materials**

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Water for human consumption comes into contact with different construction materials used in the collection, treatment, storage, and supply systems, and in the building networks up to the consumer's tap.

All the materials used in water supply structures have the potential to change water quality by leaching into the water, due to electrochemical mechanisms, biological phenomena, oxidation, corrosion, among others.

The nature of the leached compounds and the potential chemical interactions depend on the type of material used, namely, cementitious (cement, aggregates, water, additives, adjuvants, and fibers), organic (polymer matrix composites and elastomers, namely high-density polyethylene and epoxy resins) and metallic (especially stainless steel, copper, ductile iron, and metal alloys). These compounds can be harmful to human health.

The connection between the different pipework sections can be made through metal compression fittings, with the sealing being achieved by rubber sealing rings, through fittings of the same material connected by polyfusion welding.

In this work, six series of specific migrations studies from rubber materials intended for water contact have been studied. The amount of material used for each test depends on the surface-to-volume ratio (S/V) of each material and, the tests take place with a ratio S/V of 3 and 6. The experiments were carried out with ultrapure water under suitable conditions of time and temperature (1,2). The liquid-liquid extraction of 500 mL of migration water in acid and basic conditions was used for the extraction of all potential organic compounds with 200 mL of dichloromethane. The organic extract was concentrated to 0.5 mL in a Turbovap system and analyzed by gas chromatography coupled to mass spectrometry (3). A semi-quantitative method with ten deuterated standards was used for the quantification of all the compounds identified in migration waters. The electric conductivity, redox potential, color, pH, and the turbidity of migration waters were also performed.

More than 50 organic compounds were quantified in migration waters with a total concentration of up to 2608 µg/L. Most of the organic compounds showed concentrations lower than 10 µg/L, but some of them showed concentrations of up to 200 µg/L. The number of organic compounds and their concentrations decrease with the number of migration periods.

### **3.04.P-Th180 Optimisation of Solid Phase Extraction for the Determination of Bifenthrin, Estrone, and Diclofenac in Marine Environments**

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Pesticides, hormones, and pharmaceuticals are contaminants of emerging concern that can be found in many environmental samples, such as marine water. Bifenthrin (BF) is one such contaminant, which easily binds to soil particles, causing residues to be carried into the environment. The toxicity of BF to non-target organisms is of most concern, highly toxic to fin-fish and crustaceans. Low concentrations of BF have also been shown to cause behavioural effects in fish, raising concerns of neurotoxic properties in fish species, impacting the ability to forage and escape from predators. Other contaminants that are found in aquatic environments are synthetic hormones and pharmaceuticals, such as estrone and diclofenac, respectively, that do not easily breakdown in the environment.

Due to this, it is necessary to find and improve methods to extract and detect these chemicals. This research focuses on the optimisation of solid phase extraction (SPE) coupled with liquid chromatography-mass spectrometry (LC-MS). Due to the low concentrations at which these are found in marine environments, the analytes must be pre-concentrated and extracted before they can be detected using LC-MS. This project focuses on the optimisation of the pre-concentration and extraction of contaminants.

The sample is spiked with a standard solution of the analyte. Oasis HLB 6cc 200 mg extraction cartridges were used for the SPE process. During SPE the analyte is eluted using 5 mL of acetonitrile. This is then dried and reconstituted in 20/80 (% v/v)

methanol/water. An Agilent HPLC instrument was used for LC-MS/MS analysis. The columns used were a Zorbax eclipse plus C18 2.1x50 mm 1.8  $\mu$ m LC column and a Zorbax eclipse plus C18, 2.1x5 mm, 1.8  $\mu$ m UHPLC guard column at 30 °C. A 6470A triple-quadrupole mass spectrometer with electrospray ionisation (Agilent Technologies) was used for detection.

BF was detected at a concentration range of 0.065-0.079 ng/L among water samples. Estrone and diclofenac were not measured in these samples, further investigations will be carried out for these analytes. The concentrations detected may not be fatal, but an increase in concentration or repeated (chronic) exposure could have long-term damaging effects for aquatic wildlife.

To preserve marine environments it is necessary to monitor the levels of pollution in marine waters. The proposed method will enable the extraction and detection of contaminants of emerging concern.

### **3.04.P-Th181 Analysis of Pesticides, Pharmaceuticals and Personal Care Products in Drinking and Environmental Water by Direct Injection Using UPLC-MS/MS**

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The monitoring of harmful substances in drinking and environmental water is essential for protecting human health and maintaining environmental quality standards. Accurate measurements at ultra- low levels is an essential part of any trace monitoring program. As more and more pesticides, pharmaceuticals and personal care products (PPPCPs) are released into the environment, there is a growing demand for analytical methods with multiple compound groups, where one major analytical challenge for analysis lies in the wide chemical diversity of compound classes and structures.

The purpose of this work was to demonstrate a direct injection UPLC-MS/MS method for the ultra-low level determination of pesticides, pharmaceuticals and personal care products in drinking and environmental waters. The method performance study was completed on an ACQUITY Premier™ LC System with a Xevo TQ Absolute™ MS system.

A method validation study was carried out on drinking and surface water matrices. The method performance was assessed using 3 spike levels at 10, 25, and 125 ng/L for all analytes, with 6 replicates at each level. Average method performance for trueness, repeatability, linearity, and sensitivity was assessed through inter and intra-laboratory studies.

The sensitivity of the Xevo TQ Absolute™ MS system allows for the use of a direct injection approach which removes complex sample preparation and enables the simple, high-throughput analysis of pesticides, pharmaceuticals, and personal care products in drinking and environmental water.

### **3.04.P-Th182 Anthropogenic substances on Mar Menor beaches: occurrence, environmental risk, and usefulness as markers of anthropogenic contaminant sources.**

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The Mar Menor lagoon (Murcia, Spain) is one of the largest in Europe and is protected by national and international environmental legislation due to its great ecological value. However, anthropogenic contaminants such as pharmaceuticals, which are not treated efficiently in Wastewater Treatment Plants (WWTPs), could pose a risk to this ecosystem. The objective of this study was to determine the occurrence, concentrations and environmental risk of different anthropogenic contaminants on 6 representative beaches in the lagoon. Sampling was carried out in spring and summer to evaluate how seasonality influence in the measured environmental concentrations. 41 substances from 8 therapeutic groups, including parenteral compounds and metabolites were analyzed by liquid chromatography coupled to mass spectrometry with triple quadrupole analyzer. Methodological Detection Levels (MDL) are between 0.04 – 0.9 ng/L. Seasonal variation were evaluated with non-parametric test for paired samples (Wilcoxon). Risk Quotient (HQ) was used to assess the environmental risk of the substances. Laboratory chronic toxicity data were used where possible, and QSAR models were used when not available. 37 of the 41 substances were detected in at least one of the two samples, 4 of them included in the 4<sup>th</sup> Watch List of European Union. Concentrations were greatly varied between beaches and also samplings. In general, concentrations were higher in summer, but only 7 compounds were statistically significant ( $p < 0.5$ ), 4 of them were stimulants. All substances in this group were detected in 100% of the samples. The average total concentration of compounds was higher in August in all beaches except Rambla del Albujón. In May, this beach had the highest total average, while in August Santiago de la Ribera did so (984.7 ng/L), much higher than Villanitos and Urrutias (639 and 626.6 ng/L respectively). All beaches present substances posing, at least, a moderate risk. High risk ( $HQ > 10$ ) was detected for 4 compounds. Eight substances reached, at least, moderate risk ( $1 \leq HQ < 10$ ) in, at least, one of the seasons. Sixteen reached low risk ( $1 \leq HQ < 10$ ) levels in at least one location in at least one season. Caffeine, paraxanthine and carbamazepine most frequently posed a risk in the studied beaches. . This is the first study that analyzes wide variety of anthropogenic contaminants on Mar Menor beaches, as well as the influence of seasonality in their occurrence and environmental risk. It also points out the importance of including not only parent compounds but also metabolites in risk assessment as well as determine possible sources.



### **3.04.P-Th183 Chemical and Biological Transformations of ZnO Engineered Nanoparticles in Aquatic Environments: Implications for Toxicity and Environmental Risk Assessment**

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The increasing global production and use of engineered nanoparticles (ENPs) resulted in their release into the aquatic environment. In light of the potential hazard posed by emerging contaminants, extensive investigations have been conducted to elucidate their toxicity on living organisms. Notably, these studies have predominantly focused on pristine engineered nanoparticles (*p*ENPs). However, it is imperative to acknowledge that ENPs released into the environment undergo rapid and intricate physical, chemical, and biological transformations. These transformations may pivotally alter the properties of ENPs and further their fate in the environment, including toxicity. Our study aimed to assess whether chemical and biological transformations mitigate or exacerbate the toxicity of ZnO ENPs to the freshwater crustacean *Daphnia magna*. For chemical transformation, *p*ZnO ENPs were aged in solutions containing S<sup>-</sup> or PO<sub>4</sub><sup>3+</sup> ions, while to obtain the biocorona of the ENPs *p*ZnO ENPs underwent the physisorption with protein and polysaccharide. Additionally, the obtained ZnS ENPs were subsequently covered with a protein layer. The transformed ZnO ENPs (*trans*ZnO ENPs) were physicochemically characterized and evaluated in terms of acute toxicity towards *D. magna*, according to OECD Guidelines 202. Both chemical and biological transformations significantly reduced the toxicity of ZnO ENPs. Among the chemical transformations, sulphidation was the most effective in mitigating the toxic effects on *D. magna*, and in addition, the protein corona formed on previously sulphidised ZnO ENPs enhanced this effect. In conclusion, the transformations that ENPs may undergo in the aquatic environment have the potential to mitigate toxicity on living organisms. A potential mechanism for toxicity reduction may be the decreased dissolution of *trans*ENPs compared to *p*ENPs, but further studies are needed to confirm this. The results obtained may also provide a basis for the risk analysis of increasing environmental pollution by ENPs and contribute to the development of effective methods to reduce their harmful effects on the biosphere.

### **3.04.P-Th184 Improving the sensitivity of additive detection in environmental matrices using SPE and selected reaction monitoring**

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Plastic mulch film supports improved food security, particularly in regions of marginal productivity. Organic additives included within these films are not chemically bound and thus susceptible to loss to the wider soil environment prior to the breakdown of the polymer. This includes additives with potential human health impacts (e.g., phthalates), under assessment for bioaccumulation (e.g., organophosphites), and those with unknown potential hazards (e.g., oligoesters derived from biodegradable plastics). It is essential to quantify the abundance of such additives in soil, and potential uptake into plants. Testing published methods for plant matrices revealed significant interference from the plant matrix, which prohibited the detection of low-level additives. We propose that the concentration difference of soil and plant-derived compounds relative to additives currently prohibits the reliable detection of these emerging compounds of concern, particularly in settings that have recently employed plastic mulch. Resolving this is essential to determine the fate of leached additives in the early stages of plastic mulch use.

In this study we have optimised the extraction and solid phase extraction (SPE) work-up of additives from plastic mulch films. We targeted known compounds and degradation products of two commercially available mulches, a LDPE and a biodegradable (PLA/PBAT) mulch, so to better reflect the complexity of additive inputs into the environment. Fractions were subsequently analysed by GC-QTOF-MS, with increased sensitivity and exclusion of potential interference from the plant or soil matrix using selected reaction monitoring. Quantification was achieved by external calibration. This was then applied to soil, spiked with additives extracted from the film, and from a field experiment where the mulches had been used for three sequential growing seasons.

The sequential aminopropyl-silica SPE following microwave extraction yielded two to three fractions of interest per mulch type, and reduced the interference of the soil/plant matrices, to allow reliable detection of additives in spiked soils with high recovery (>90%). This approach will then be applied to soils that have been exposed to plastic mulch films for three growing seasons, where theoretical loading from the plastic mulch use over three years is in the nanogram range per gram of soil, excluding the degradation of additives.

### **3.04.P-Th185 Occurrence of pharmaceuticals in surface water from rural areas located in the Northwest of Spain**

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Urban wastewater constitutes a relevant vector of pharmaceuticals in the aquatic environment. Many of these compounds are incompletely removed in the sewage treatment plants (STPs), and thus reaching surface watercourses and reservoirs, and even groundwater. In remote and rural areas, domestic wastewater receives limited treatment and attention, which added to leaks from sewers and septic tanks, may contribute to the contamination of surface and groundwater with anthropogenic substances. Galicia, in the Northwest of Spain, is characterized by a very dispersed population all over the territory. Thus, monitoring the presence of pharmaceuticals in surface and groundwater is required to understand their distribution in the environment, to identify markers of anthropogenic pollution, and to assess their potential eco-toxicological risks.

The aim of this presentation encompasses i) to develop and validate a fully automated methodology based on SPE online connected to LC-MS/MS for a set of pharmaceuticals commonly reported in urban wastewater; ii) to investigate the temporal fluctuations of the selected compounds in rural dispersed population areas of Galicia through four sampling campaigns, performed in two main rivers in the area.

SPE online system, based on a reusable and reversed-phase sorbent, tailored to liquid chromatography mass spectrometry (LC-MS/MS) provided a sensitive and accurate determination of a wide selection of pharmaceuticals in water samples through easy sample processing. Pharmaceutical residues were ubiquitous in the investigated samples. Tramadol, o-desmethyl venlafaxine, irbesartan, valsartan acid and olmesartan are proposed markers of municipal wastewater discharges. Among them, olmesartan is highlighted as potentially hazardous in the aquatic environment attending to currently available PNEC values with risk quotients of 4.4 and 254 in reservoir and river water, respectively.

### **3.04.P-Th187 Marine ecotoxicity of amines used as solvents in carbon capture processes: Species sensitivity distributions and additive toxicity aspects.**

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2-Amino-2-methyl-1-propanol (AMP) and piperazine (PZ) are amines used as solvents in carbon capture (CC) technologies, and during this process some of the utilized amines will be emitted to the atmosphere. With rapid development and implementation of amine-based technologies with the purpose to decarbonize industries and meet climate goals, there is a concern for potential environmental impacts of these emitted amines. To assess their environmental risk, information is needed about their environmental fate and ecotoxicity, however, surprisingly little is known about the ecotoxicity of AMP and PZ. As the ocean represents the sink for most anthropogenic emissions, we investigated ecotoxicity thresholds for AMP and PZ in three marine species representing three trophic levels, namely the marine algae *Skeletonema pseudocostatum*, the copepod *Calanus finmarchicus* and fish Atlantic cod (*Gadus morhua*). Acute toxicity thresholds (72 h-EC<sub>50</sub>, algae, and 96h-LC<sub>50</sub>, copepod) were in the range 159-522 mg/L and 147-381 mg/L for AMP and PZ, respectively. For Atlantic cod, which were exposed as developing embryos in the egg, observations up to 8 days post exposure did not provide evidence for delayed mortality or larvae deformations. We also tested binary mixtures of the amines on the copepod, which was the most sensitive species, and found that the amines displayed a pattern of additive toxicity. Furthermore, we collected literature data for both amines and a range of degradation products to assess species sensitivity distributions. Our data are important for regulatory agencies and policy makers involved in environmental risk assessment processes relating to amine-based CC technologies highlighting the needs for testing towards organisms at different trophic levels.

### **3.04.P-Th188 Spatial variability of microplastic concentration in benthic sediments from coastal aquaculture region of Korea using a new device for extracting microplastics from sediments**

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To date, methods for extracting microplastics from marine sediments have some limitations such as complexity, low extraction efficiencies and incompatibility with very fine sediments. Here, we developed an improved method to extract microplastics from benthic sediments using the principle of density floatation in a single step with a mean efficiency of 99.5% ( $\pm$ SE 2.25%; min 90%; max 100%). We used the method to analyze the spatial distribution of microplastics in benthic sediments from 7 coastal aquaculture regions of Korea. The abundance of microplastics in benthic sediments ranged from 578 to 3,228 particles/kg, and the fragment was the most dominant shape. Polypropylene and polyethylene, and polystyrene accounted from 20~69%, 14~34%, and 0~29% of total microplastics, respectively. The distribution of microplastics in benthic sediments peaked in the 100~200  $\mu$ m size range.

### **3.04.P-Th189 A study of chemical toxic effects on juvenile Rockfish (*Sebastes schlegeli*) exposed to hull cleaning discharge using brain and liver transcriptome analysis**

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Antifouling paints serve as protective coatings on vessels, preventing the attachment of marine organisms. Chemical constituents of antifouling paints can be released into the marine environment during vessel cleaning processes, including in-

water cleaning (IWC). During the hull IWC process, a mixture of antifouling paint particles is produced and directly discharged into the coastal environment, yet few studies have investigated their toxic effects on resident marine species. Environmental IWC discharges serve as a source of toxic substances, including metals and biocides that may leach from them, potentially causing harmful effects on non-target organisms residing in the area. In this study, the toxic effects of IWC discharge on juvenile rockfish (*Sebastes schlegeli*) which is one of the major fishery resources was investigated. Chemical analysis revealed that zinc ( $8.05 \pm 0.96$  to  $189.96 \pm 47.76$   $\mu\text{g/L}$ ), followed by copper ( $0.87 \pm 0.19$  to  $1.97 \pm 0.60$   $\mu\text{g/L}$ ), was the most abundant compound found in the IWC discharge. No mortality was observed in any of the experimental groups at 7 days after exposure. However, variation in reactive oxygen species (ROS) and acetylcholinesterase (AChE) activity was noted in juvenile rockfish exposed to a 10-times dilution of IWC discharge. Genes associated with oxidative stress, detoxification, and the immune response exhibited significant alterations after exposure to the 10-times dilution-IWC discharge. Transcriptomic variations in the brain and hepatic of juvenile rockfish exposed to IWC discharge also revealed changes in genes related to the immune system and detoxification. Furthermore, significant alterations in the nervous system development and homeostatic processes were also observed in the brain-transcriptomic network analysis. The findings of this study contribute to an enhanced understanding of the effects of zinc-abundant in-IWC discharge on the neuronal and immune toxicity of commercially important fishery species.

**3.04.P-Th190 Acute toxic effects of hull in-water cleaning discharge on embryonic flounder (*Paralichthys olivaceus*)**  
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Antifouling paints, consisting of biocidal compounds, are applied to boat and ship hulls to prevent or minimize the attachment of fouling organisms. Despite the potential toxicity risk associated with these pollutants, there is a limited number of studies investigating and monitoring the toxic effects on embryonic fish using IWC discharge collected from multiple ships. In this study, hull IWC discharge from four ships were collected in 2022 and 2023 and toxic effect assessments were conducted on flatfish fertilized embryos. After dividing the IWC discharge into untreated discharge and filtered wastewater, fertilized embryos were exposed based on various dilution factors (5-, 10-, 100-, and 1000-fold dilutions). As a result of chemical analysis of IWC discharge, Fe, Cu, and Zn were confirmed in high proportions. The experimental groups exposed to hull IWC discharges exhibited higher mortality rates at lower dilution folds compared to those at higher dilution folds. Moreover, the mortality rate was high in embryonic fish exposed to untreated discharge compared to filtered discharge. Malformations in morphogenesis, including pericardial edema, dorsal curvature, tail fin fold defects, and developmental delays, were observed in fertilized embryos following exposure to IWC from all four different ships. To understand the molecular biology of malformation, eight genes (heart formation (*nkx2.5*, NK2 transcription factor-related 5; *SOX6*, SRY-box-containing gene 6; *robo1*, roundabout receptor1), bone malformation (*bmp4*, bone morphogenetic protein 4), fin malformation (*plod2*, procollagen-lysine 2-oxo-glutarate 5-dioxygenase 2, *furin*, furin; *wnt3a*, Wnt family member 3a), and tumors (*TP73*, Tumor protein p73) related to morphogenesis were evaluated using qRT-PCR. To clarify the potential toxic effects of IWC discharge, we also conducted RNA-seq (high-throughput sequencing) on embryonic flounder exposed to hull IWC wastewater. This study provided crucial evidence of the risks associated with IWC discharge when exposed to marine organisms. Taken together, these results may inform strategies to improve hull-cleaning wastewater pollution management to better protect coastal ecosystems.

**3.04.P-Th191 Occurrence and seasonal variations of 160 current-use pesticides in surface seawater of Korean coastal waters**

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With the frequent used of chemical pesticides, the current-use pesticides (CUPs) emerge and concentrate in the sea. However, there is little research on this topic. The present study, 160 CUPs were screened in 66 seawater samples collected from two coastal bays, with high agricultural activities. The number and total concentration of detected CUPs were much higher in summer than in winter season, which was due to their large application in the local agriculture activities. In studied two bays, higher detection and concentration of CUPs were found in Gunsan Bay than Jinhae Bay, indicating intense human activities and riverine input. In summer, 73 pesticides in seawater samples were detected and total concentration of the pesticides ranged from 5,140 to 287,000 ng/L. Higher levels showed in herbicides than in insecticides and germicides, and mean concentrations of metazosulfuron, imazosulfuron, mefenacet and benxulfuron-mehty were higher than 1,000 ng/L. The germicides with relative high levels were isoprothiolane, tebuconazole, tricyclonazole, hexaconazole, and azoxystrobin. Diuron (herbicide and antifouling paint biocide) and DEET (mozzie repellent) showed 100% detection frequency and high concentration. These detected pesticides in seawater were all much lower values than the toxicological endpoints, presenting low toxic effects to aquatic organisms.

**3.04.P-Th192 Comprehensive monitoring of emerging contaminants following “Daniel” and “Elias” storm events at Pagasitikos Gulf, Eastern Mediterranean Sea, Greece, utilizing the technique of LC-VIP-HESI-TIMS-HRMS**

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Comprehensive monitoring of emerging contaminants provides insights on the quality of ecosystems. Chemicals such as plant protection products, pharmaceuticals and per- and polyfluoroalkyl substances (PFAS) often end up in the environment and are distributed in different compartments. Their presence may be attributed to human related activities and wastewater treatment plants' (WWTP) inability to remove them from wastewater stream. Upon reaching the sea, biotic and abiotic processes take place, that produce transformation products (TPs), suspected to cause more potent effects than parent compounds towards the environment and human health. Thessaly, Greece, was severely struck by sequential major storm events "Daniel" and "Elias" during the fall of 2023. Approximately 1.5 million tons of water per square kilometer rained down the surrounding area during the phenomena. Such events caused by natural induced environmental change may increase the number of chemicals ending up in the marine ecosystem. Aiming to extract as many contaminants as possible, generic sample preparation protocols were applied using multilayer mixed-mode SPE cartridges to enrich final extracts with thousands of non-volatile, thermally unstable, semi-polar to polar organic pollutants. The analytes were separated using a Liquid Chromatography system linked to a hybrid Trapped Ion Mobility Spectrometer coupled to High Resolution Mass Spectrometer (LC-TIMS-HRMS). The occurrence of more than 2,500 chemicals from different chemical classes was investigated via wide-scope target analysis. TIMS provides an additional dimension of separation, adding increased value of confidence to the identification criteria, minimizing false positive selection, further optimizing target screening methodology. Preliminary results indicate the presence of numerous plant protection products in seawater samples, such as Azoxystrobin and Atrazine along with their TPs: Azoxystrobin acid, 2-hydroxy-atrazine, desethyl-atrazine and desisopropyl-atrazine, pharmaceuticals, in areas close to WWTPs, such as Carbamazepine and its' TPs: 10,11-epoxy-carbamazepine and 10-hydroxy-carbamazepine, possibly due to overflowing of said facilities. The presence of PFAS like Perfluorooctanesulfonic acid (PFOS) and Perfluorooctanoic acid (PFOA) is also confirmed, linked to the destruction of port infrastructure. This work was funded by the Ministry of Development & Investment of Greece, National Strategic Reference Framework.

### **3.04.P-Th193 Assessing the Removal and Risks of Micropollutants and Transformation Products in PAC-AGS Treatment**

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The removal of micropollutants poses a challenge in conventional biological reactors (BR), leading to the formation of transformation products (TPs) and potential risks to ecosystems. Addressing these challenges requires new technologies. In this study, we applied powdered activated carbon-aerobic granular sludge (PAC-AGS) technology to a conventional BR to treat micropollutants and their TPs. Extensive LC-HRMS screening was conducted to identify and quantify substances occurring in wastewaters before and after the treatments. Samples were collected from the influent and effluent (retention time: 15hr) of the BR at a domestic sewage treatment plant. The effluent was collected after treating stage 1 (AGS) for 4 hours and stage 2 (PAC-AGS) for either 3 or 6 hours. Target screening quantified 72 substances (pharmaceuticals: 34, pesticides: 18, industrial: 5, TPs: 15) among 219. Of these, 61 were confirmed in the influent, and 47 (including 10 TPs) were confirmed in the effluent. When only the first stage (AGS) was applied to the influent, 57 substances (including 13 TPs) were confirmed. In contrast, when the second stage (PAC-AGS) was applied, 16 substances (including 1 TP) were confirmed. The number of substances detected in PAC-AGS decreased threefold compared to the conventional BR, with the total concentration decreasing sevenfold in PAC-AGS (1,400 ng/L) compared to the conventional BR (9,500 ng/L). In the effluent, the highest concentrations were found in the following order: valsartan acid (2,500 ng/L), irbesartan (780 ng/L), olmesartan (640 ng/L), Tris(2-butoxyethyl) phosphate (TBEP) (600 ng/L), and lidocaine (550 ng/L). Compared to the removal rates of the conventional BR, valsartan acid decreased by 81%, irbesartan by 100%, olmesartan by 54%, and both TBEP and lidocaine by 95%. The risk quotient (RQ) values of four substances (n-desmethyl tramadol, diclofenac, carbamazepine, venlafaxine), originally posing potential environmental risks with an RQ value of 1 or higher, were reduced to 0. Overall, the RQ estimate decreased from 190 (conventional BR) to 77 (PAC-AGS), confirming that the risk of residual substances during PAC-AGS treatment is reduced by more than two times compared to conventional BR treatment. These results affirm that PAC-AGS technology is more effective in removing micropollutants and reducing TP formation compared to conventional BR, ultimately decreasing the risk to aquatic organisms.

### **3.04.P-Th194 RHE-MEDIation Lighthouse: Responsive hub for long term governance to destress the Mediterranean Sea from chemical pollution**

**Rallis Lougkovois<sup>1</sup>, Constantinos Parinos<sup>2</sup>, Georgios Gkotsis<sup>3</sup>, Maria-Christina Nika<sup>4</sup>, Alexandra Pavlidou<sup>2</sup>, Nikolaos S. Thomaidis<sup>4</sup>, Ioannis Hatzianestis<sup>2</sup>, Stylianos Samios<sup>5</sup> and Georgios Katsouras<sup>5</sup>,** (1)Hellenic Center for Marine Research (HCMR), Greece, (2)Hellenic Centre of Marine Research, (3)Chemistry, National and Kapodistrian University of Athens, Greece, (4)National and Kapodistrian University of Athens, Greece, (5)Athens Water Supply and Sewerage Company S.A.

Restoring and protecting the Mediterranean Sea from chemical pollution is one of the most urgent challenges of our time. That is because today, every stage of 'source to sea' applies heavy pressure to the entire water cycle. Decades of pollution and unsustainable energy use have severely degraded the quality of marine ecosystems. Major pressures include discharge, loss and leakage of chemicals. Plant protection products, pharmaceuticals, heavy metals and radioactive substances primarily originate from land-based sources, while marine transport and sea-based activities also contribute. Contamination is highest in coastal waters and toxic substances accumulating in seafood affect humans through the food chain. Ocean restoration is pivotal to the implementation of the United Nations' 2030 agenda for sustainable development, while the EU's implementation plan to restore waters by 2030 has paved the way for the International Ocean Governance 2022 agenda, a milestone for ocean governance. The new Horizon Europe project "RHE-MEDIation" supports the work of policy makers by providing advanced,

ready-to-use remediation solutions for Mediterranean hotspots and a toolkit for monitoring and control, enabling the engagement of civil society at the same time. To characterize and prioritize emerging contaminants, additional research concerning major sources, transport pathways in open or deep-sea settings, determination techniques and adverse effects is required. Upon reaching the marine environment, many compounds are subjected to biochemical processes that produce transformation products, often more potent in terms of adverse impact to the environment and to human health than the parent compounds. Comprehensive monitoring of emerging contaminants demands wide-scope screening analytical techniques, which in turn provide better understanding about the chemical imprint of related anthropogenic activities. Scientists and scientific groups that take part in organizing strategies for the preservation of the Eastern Mediterranean aquatic ecosystem are also heralds of disseminating information resulting from related research. The RHE-MEDIATION project also aims to enable knowledge transfer at national and international level by making holistic approaches in the field of organic geochemistry. This work has received funding from the European Union's HORIZON EUROPE Research and Innovation Programme under the Grant Agreement No. 101113045.

#### **3.04.P-Th195 Exploring the Potential of Vacuum-Assisted Evaporation Concentration for Improved Analysis of Very Polar Compounds: Comparing Analysis Platforms, Method Development and Method Validation**

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Studies reviewing the efficiency of current water analysis have pointed out a severe lag in analytical techniques covering very polar compounds. Especially persistent and mobile organic chemicals (PMOCs) which are known to be toxic to the environment and to humans. Therefore, there is a pressing need for the development of analytical methods able to analyse very polar compounds. This study developed and validated a vacuum-assisted evaporation concentration (VEC) as a water sample preparation method, with a particular focus on very polar compounds, such as PMOCs. The efficiency of the VEC method was subsequently compared to multiple-layer solid-phase extraction (SPE). Moreover, its compatibility with different chromatographic techniques was evaluated, including supercritical fluid chromatography (SFC) and reversed-phase liquid chromatography (RPLC). The developed VEC method, allowed the rapid evaporation of 100 mL samples. Of two tested evaporation endpoints, dryness and a residual volume of 0.3, evaporation to dryness was regarded as the preferred method due to the potential variability induced by salt deposition in residual volume evaporation. The high salt contents were also suspected to cause strong signal suppression in the applied RPLC-MS/MS analysis. The VEC recovery assessment resulted in recoveries spanning 40% to 60% for almost all compounds. In comparison to SPE, VEC resulted in overall lower recovery, but achieved higher VEC recovery for targeted very polar compounds. In conclusion, VEC has the potential to analyse very polar compounds including PMOCs. Further research is needed to determine the best analysis strategy for VEC concentrated samples, in order to further optimise and fully understand its capabilities.

#### **3.04.P-Th196 Exploring the Absorption Dynamics of Benzophenone-3 and Octocrylene in Polyethylene and Polypropylene in Pure and Sea Water**

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Benzophenone-3 (BP-3) and octocrylene (OC), commonly present in skincare products for UV protection, pose environmental risks when entering the ocean through activities like swimming or sewage discharge. Detected in marine environments, these compounds, known to bioaccumulate in aquatic organisms, contribute to issues like coral bleaching and tissue necrosis. Additionally, they could adsorb onto microplastics (MPs) which are likewise prevalent in marine environments. This study investigates the absorption capacity of polyethylene (PE) and polypropylene (PP) when exposed to BP-3 and OC, using a fractional factorial design for efficient resource utilization in experimentation.

The controlled experiments exposed the materials to both pure and seawater. The synthetic seawater serves as a simulation of a natural environment, providing a more realistic assessment of the absorption process. The experiments were performed by analysing the exposure solution over time, using both High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) and Voltammetric determination (VD) using commercial screen-printed electrodes. HPLC-MS/MS is a powerful tool for the detection and quantification of analytes, providing high sensitivity, selectivity, and accuracy, while VD offers a straightforward working protocol and ease of miniaturization, making it an appealing tool for measuring contaminants of emerging concern even with the capability to perform on-site analysis.

The primary focus of this work is to assess the absorption capacity of PE and PP, providing valuable insights into their potential as both vectors of transport and mediums for the removal of these contaminants from water systems. A fractional factorial design was employed considering variables including temperature, salinity, exposure time, and pH. These selected variables encompass a diverse range of factors critical for assessing real-scale variations in the environment and understanding the absorption capacity of BP-3 and OC onto MP surfaces. The results of this study could have significant implications for the development of more effective and sustainable methods for water treatment and pollution control, as well as real-time on-site analysis with voltammetry. This research contributes to the broader understanding of the interactions between synthetic polymers and organic contaminants and could inform future strategies for environmental protection and waste management.

### **3.04.P-Th197 The Occurrence of Poly- and Perfluoroalkyl Substances (PFASs) and Potential Sources in the River Liffey, Ireland**

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Decades of widespread use of poly- and perfluoroalkyl substances (PFAS) globally and in Ireland has raised concerns about potential legacy environmental contamination and their impact on human health. PFAS can enter the environment during manufacturing of substances, use of the substances themselves and through disposal. The persistence, bioaccumulation, toxicity and long-range transportation of PFAS compounds has meant that they are ubiquitous in the environment and have been detected globally in several sample types (soil, air, biota and water).

Recent research in Ireland has indicated the presence of PFAS in a variety of media, including surface water. Therefore, the main objective of this work was to characterise exposure risks to the environment and human health. 20 surface water samples were taken from sites along the river Liffey, between a tributary in Kildare to the sea. It was found that concentrations of up to 2 µg/L of individual PFAS compounds were detected along the river, with highest concentrations towards the sea.

To interpolate the nature and distribution of PFAS compounds along the river and identify potential links to upgradient sources, a 4-step hierarchical process was used.

1. Geographical proximity - The river Liffey flows through the centre of Dublin and is known to have legacy contamination. Known primary and secondary sources of PFAS in the catchment include civil (airports and fire stations), anthropogenic (wastewater treatment facilities and waste facilities) and industrial pressures (chemical manufacturing plants, paper and wood processing facilities and other industries with emissions licences).
2. Chemical footprint - Nowadays, there are 4700 identifiable PFAS compounds. Individual compounds have unique properties and different combinations are used by industries for specific purposes. By identifying unique compounds and distinguishable compound ratios, a specific diffuse PFAS source can be inferred.
3. Dimensional calculation – A solute transport model was employed to predict the spatio-temporal fate of PFAS and infer proximity to industrial sources.
4. Dimensional modelling and cluster analysis was employed to refine the source identification.

In conclusion, with the data obtained the occurrence of different PFAS substances were linked to potential civil, anthropogenic and industrial sources in the River Liffey catchment, remarking the effect of the emission not only in the near environment but also in a further one.

### **3.04.P-Th198 An approach to the presence of the recent emerging organic contaminants adsorbed onto microplastics in the Canary Islands.**

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Microplastics have traditionally been recognized as significant pollutants in marine environments. Their capacity to adsorb different organic substances, thereby serving as vectors of contamination, has been extensively researched. Numerous studies support this assertion. In our investigation, an approach to the occurrence of the most recent organic compounds from the Watch List compounds, in accordance with the criteria set by the European Union, adsorbed on microplastics was studied at the coastal areas of The Canary Islands.

For that, an ultrasound-assisted extraction method was optimized and employed as extraction process. The influence of the various factors affecting the extraction process was investigated through an experimental design with Minitab® software. This approach allowed us to evaluate the individual impact of each variable on the extraction process and the identification of potential correlations among these variables.

Following the extraction process, an ultrahigh-performance liquid chromatography system coupled with a triple quadrupole mass spectrometer (UHPLC-MS/MS) was used for the separation and detection of analytes. Several validation parameters were tested to determine the suitability of the proposed method for the compounds of interest.

Finally, the novel methodology was applied to analyze samples of microplastic fragments and plastic pellets gathered from various tourist and secluded beaches of Tenerife in the Canary Islands archipelago (Spain). The objective of this assessment is to ascertain the presence of these emerging contaminants adsorbed on the microplastic residues.

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### **3.04.P-Th199 In-Depth Investigation of Organic Micropollutant Burden in the Dnieper River Basin Using HRMS-Based Workflows**

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Organic micropollutants are constantly released into the environment due to various anthropogenic activities. The final receiver of these pollutants is usually the freshwater ecosystem, thus degrading the water quality and harming the organisms that live in these habitats. The occurrence of organic micropollutants has been globally reported in freshwater bodies, including sorption in sediments based on their physicochemical properties, findings that highlight their potentially harmful effects on environmental and human health. Consequently, monitoring studies on surface water and sediment samples are crucial for assessing the environmental quality standards and identifying the river basin specific organic micropollutants and their concentration levels.

The aim of this study is the in-depth investigation of organic micropollutant burden in the Dnieper River basin which belongs to the fourth longest river in Europe. In this framework, 12 surface water samples and 8 sediment samples were gathered from the Dnieper River in Ukraine during 2023. The sample analysis utilized complementary chromatographic techniques and ionization modes, coupled with high-resolution mass spectrometry (LC-ESI-QToF MS and GC-APCI-QToF MS). The data treatment consisted of wide-scope target screening protocols investigating the presence of more than 2,500 organic micropollutants from various categories, such as pharmaceuticals, per- and polyfluoroalkyl substances (PFAS), industrial chemicals and plant protection products, and their transformation products (TPs), as well as the suspect screening for 65,000 environmentally relevant organic micropollutants following suspect screening methodologies.

Preliminary results highlight the occurrence of industrial chemicals (benzotriazole, tolytriazole), pharmaceuticals (aceclidine, carbamazepine) stimulants (caffeine, nicotine, and the TP cotinine), and plant protection products (atrazine and the TP atrazine-desethyl, metolachlor). In addition, the results of this study were compared with the data gathered from the previous sampling campaign on the Dnieper River in 2020.

### **3.04.P-Th200 A comparative study of the presence of organic contaminants of emerging concern in two urban wastewater treatment plants from Canary Islands (Spain) and Tunisia.**

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The role of quality monitoring in municipal wastewater is pivotal for sustainable development. Wastewater treatment plants (WWTPs) play a crucial role as the primary source of emerging pollutants released into the environment and their persistence is often a challenge for conventional treatments. These emerging pollutants pose significant issues to water management due to their potential and definite environmental impact as well as potential risks to human health. To better understand their fate in the environment, monitoring systems must be established to ensure the acquisition of representative data regarding their presence.

This study focuses on the comprehensive analysis of wastewater samples obtained from two municipal WWTPs located in Las Palmas de Gran Canaria (Spain) and Mahdia (Tunisia), to identify and quantify emerging contaminants included in the lasted listed in the European Union (EU) Watch List.

In this way, an optimized solid phase extraction method was employed for the extraction process. Afterward, an ultrahigh-performance liquid chromatography system coupled with a quadrupole Time of Flight mass spectrometer (UHPLC-QToF) was utilized to detect and determine the analytes. Validation parameters were assessed to ascertain the appropriateness of the proposed method for the compounds of interest.

Water samples from both the influent and effluent of the two WWTPs studied were analyzed, and the obtained results were subsequently compared. This research contributes valuable insights into the presence of emerging contaminants in municipal wastewater systems.

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### **3.04.P-Th201 Determination of Glyphosate, Aminomethylphosphonic Acid (AMPA), and Glufosinate in Drinking Water Using Direct Analysis by Liquid Chromatography Tandem Mass Spectrometry**

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Reliable analytical methods are needed for detection, quantification, and identification of glyphosate, aminomethylphosphonic acid (AMPA), and glufosinate in drinking water. Accurate and reliable methods are required to monitor these widely used pesticides in drinking water from different sources. This work describes a simple direct analysis method, based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), which avoids the need for lengthy and laborious

derivatization and solid-phase extraction (SPE). Samples of water were injected directly into the LC-MS/MS system (ACQUITY™ Premier UPLC™ System with Xevo™ TQ Absolute Tandem Mass Spectrometer) using the Anionic Polar Pesticide Column. The performance of the method was successfully evaluated in three different types of drinking water. The extremely high sensitivity of the Xevo TQ Absolute System was demonstrated with reliable detection for all three analytes at concentrations as low as 10 ng/L (0.01 µg/L). The performance of the method was evaluated in-house and by an inter-laboratory study using spiked water samples. Results from both studies showed that the trueness of the method was between 82 and 110%. Close agreement was observed with the repeatability, within laboratory reproducibility, and between laboratory reproducibility, all being <16% RSD. The method is considered sensitive, specific, accurate, and suitable for the determination of these challenging analytes in a range of different types of drinking water, for checking compliance with regulatory limits, and to support studies focusing on human exposure.

### 3.04.P-Th202 Improved compound identification in GC-MS analysis using an EI&CI-TOFMS

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A time of flight (TOF) mass spectrometer simultaneously operating an electron ionization (EI) and a chemical ionization (CI) source is presented for non-target analysis. By coupling both ionization sources directly to one single gas chromatograph (GC), structural as well as accurate mass molecular ion information is generated simultaneously from each analyte. Hence, target and suspect screening analysis as well as effective non-target analysis using GC-MS is improved considerably. In this poster, various experiments will be discussed, illustrating the potential of the GC-EI&CI TOFMS especially for non-targeted approaches, including applications in fields such as environmental contaminants, material emissions, food and flavor analysis and metabolomic research.

An Agilent 6890A GC was coupled to an EI&CI TOFMS (ecTOF, TOFWERK, Thun, Switzerland), using a 70 eV EI source and the newly developed ToFwerk HRP CI source. Various GC methods and sampling procedures were employed depending on the analytical need of the study, including liquid injection of extracted samples, headspace sampling including SPME and thermal desorption using Tenax tubes. To generate the ideal molecular ion information different reactant ions (e.g., [NH<sub>4</sub>]<sup>+</sup>, [N<sub>2</sub>H]<sup>+</sup>, [H<sub>3</sub>O]<sup>+</sup>) were used for the chemical ionization process.

Standard procedures employed by routine laboratories, e.g., target screening for (material) emissions or detection of unknown volatile constituents in consumer products and within the environment, are shown to be feasible using the GC-EI&CI TOFMS. Whilst standard methods mainly focus on target analysis, suspect screening is improved and non-target analysis enabled using the GC-EI&CI TOFMS. Especially when EI library hits are fair with low corresponding probability, chromatographic and CI information can be used to increase compound identification confidence. False positives from an EI only approach can easily be identified and often correctly annotated using the additional information from the GC-EI&CI TOFMS. Furthermore, compounds that are not listed in libraries have a high uncertainty for identification using an EI only approach. Using the accurate mass information generated by CI, molecular sum formula for these unknowns can be derived. Combining this with the structural information generated by EI, tentative structure elucidation becomes feasible in many cases. Hence, non-target approaches become viable using the GC-EI&CI TOFMS.

### 3.04.P-Th203 Tidal influence on the distribution and partitioning of pharmaceuticals in estuarine environments

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Coasts are the interface between land and the ocean, the vastest ecosystem on Earth. They are undergoing tremendous socioeconomic and environmental changes due to increasing utilization and development. Pharmaceutically active compounds (PhACs) have been described as “pseudo-persistent” pollutants due to their continuous release and may present a threat to marine ecosystems. Estuaries are very dynamic environments, concentrations of contaminants are strongly affected environmental factors. This work focuses on the study of spatio-temporal variations in the concentrations of a wide range of PhACs and other sewage-derived contaminants during tidal events in several estuaries from SW Spain. Different economic activities take place at these sites (Guadalquivir, Guadalete, and Barbate estuaries), including aquaculture, maritime traffic, and wastewater discharges from industries and urban areas.

Water (n = 120) and surface sediment samples (n = 50) were collected including sampling of tidal events. Sediment samples and suspended solids resulting from filtering of aqueous samples underwent pressurized liquid extraction (PLE). Those extract and water samples were collected to use a multiresidue method based on solid-phase extraction (SPE), followed by ultra-performance liquid chromatography-triple quadrupole spectrometry (UPLC-MS/MS).

Thirty-seven out of 109 compounds were detected in our samples, with Sucralose, Fenoprofen, Gemfibrozil, and Caffeine accounting for the highest frequency of detection (>80%) and concentration (>10 ng mL<sup>-1</sup>). Concentrations of PhACs were usually higher in upstream stations with respect to the stations located closer to the mouth of the river. A conservative behavior was observed for many of them, such as carbamazepine, which showed a linear decrease in relation with salinity changes along the estuaries and within tidal cycles. Those concentrations were clearly influenced by tidal events, with values rising during low tides and decreasing during high tides due to the dilution with cleaner ocean water.



PhACs were also detected in suspended solids and surface sediments at higher concentrations ( $\text{ng g}^{-1}$ ) than in the aqueous samples. Partitioning was established by determination of water/sediment and water/suspended solids ratios. Such ratios were directly related with the hydrophobicity of the different target compounds ( $\log K_{ow}$ ), but no significant changes were detected along the estuary and within tidal cycles.

### **3.04.P-Th204 Factorial Design Optimization of a Bioanalytical Method Using Low-Temperature Purification Extraction (LTPE) and LC-MS/MS to Determine Levamisole in Fish Meat**

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Aquaculture has been the fastest-growing food production sector in the world. However, the spread of diseases is one of the biggest limitations of this system, mainly due to the reduced number of regulated drugs. In this scenario, levamisole, an anthelmintic and immunostimulant drug, has been studied as a promising alternative for fish farming. Investigations of drug residue depletion in the target animals are demanded for medicines aimed at food-producing animals. For so, the availability of high-sensitivity and selectivity analytical methods for the determination of drug residues at trace concentrations in fish meat (muscle plus skin in natural proportion) is underlying, which is highly critical when considering complex matrix such as fish fillet. For so, this study aimed to optimize a novel bioanalytical method following a sample preparation involving a low-temperature purification extraction (LTPE) procedure associated with analysis by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) for determination of levamisole residues in fish meat. A fractional factorial design was employed to select the variables which affected the sample preparation procedure. The results showed that the addition of ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) in the extraction phase, amount of primary secondary amine (PSA) in the clean-up, centrifugation temperature, and centrifugation time were statistically significant at the tested levels. To determine the best extraction conditions, a final optimization of these significant parameters was performed by using a central composite design (CCD). The optimum conditions for levamisole extraction by LTPE were achieved by using 1.0 g of sample, 2.0 mL of acetonitrile containing 1%  $\text{NH}_4\text{OH}$ , freezing time of 30 min, centrifugation at 2,900  $\text{xg}$  for 2 min at  $-5^\circ\text{C}$ , and clean up step with 100 mg of PSA. The LC-MS-MS analysis was conducted with a mobile phase composed of methanol and water, both with 0.5% formic acid under isocratic elution (85:15 v/v). The electrospray interface source was set to operate in positive mode. Therefore, multivariate optimization allowed us to obtain a bioanalytical method with high efficiency (extraction efficiency > 90%), adequate selectivity and detectability ( $\text{LOQ} = 1 \text{ ng g}^{-1}$ ) for levamisole quantification at residual concentrations (1-100  $\text{ng g}^{-1}$ ) in fish meat.

### **3.04.P-Th205 Occurrence and Trophodynamics of Organic UV Filters, Benzotriazole UV Stabilizers and Aromatic Secondary Amines in the Food Web of the St. Lawrence Estuary Beluga Population.**

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Organic UV filters (UVFs), benzotriazole UV stabilizers (BTZ-UVs), and aromatic secondary amines (Ar-SAs) are contaminants of emerging concern in the environment that may cause adverse health effects in organisms. A previous study detected some of these contaminants in the blubber and liver beluga (*Delphinapterus leucas*) from the St. Lawrence Estuary (SLE), a population that is considered endangered in Canada. However, the trophodynamics of these contaminants in the food web of SLE beluga is currently unknown. To fill this knowledge gap, tissues were collected from 17 known and potential prey ( $n=183$  including 15 fish species and 2 invertebrates) of SLE beluga in 2019 and 2020 to investigate the biomagnification patterns of selected UVFs, BZT-UVs and Ar-SAs in the SLE beluga's food web. Carbon sources and trophic position of all beluga preys items were determined using stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope ratios, respectively, and used to identify most probable dietary sources based on their respective contaminant loads. Contaminant levels, which were determined using gas chromatography and mass spectrometry (GC-MS/MS), showed decreasing concentrations from UVFs, to BZT-UVs and Ar-SAs. Except for BP, all UVFs were frequently detected (>50%) in the 17 prey species. The most common compounds detected were five UVFs (BP3, EHMC, EHS, HMS, OC) and four BZT-UVs (UV326, UV320, UV328 and UV327). Of the target contaminants with 100% detection in the 17 prey species, UVP, UV328, total BZT-UVs, HMS, BP3, and total UVFs were positively associated with  $\delta^{15}\text{N}$ , indicating that these contaminants were biomagnified in the SLE food web. Although mean levels of EHS and OC that were analyzed were not associated with  $\delta^{15}\text{N}$ , positive correlations were identified when considering all individuals in the analysis, suggesting that EHS and OC may also biomagnify in the SLE food web. UV327 and EHMC were found in all prey samples but were uncorrelated with  $\delta^{15}\text{N}$ . This study provides a baseline for monitoring these contaminants in the SLE beluga food web and contribute to a better understanding of the environmental fate of these contaminants in the estuarine environment.

### **3.04.P-Th206 Atmospheric Pressure Ionization GC/MS/MS of Multi-Class Semivolatile Organic Compounds: Reducing Environmental Impact Without Compromise**

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The analysis of multi-class, semivolatile organic compounds (SVOCs) in environmental matrices is a common analysis in labs around the world. Therefore, modernizing SVOC analytical methods has the potential to reduce the environmental impact that labs themselves have on the environment. An important aspect of reducing the environmental impact of SVOC methods is reducing solvent consumption associated with sample preparation. Gas chromatography atmospheric pressure chemical ionization (GC-APCI) produces intense molecular ions for most analytes. When combined with tandem quadrupole MS/MS then, the sensitivity of GC-APCI combined with MS/MS directly contributes to the improved environmental sustainability of SVOC analytical methods. Furthermore, GC-APCI is readily adaptable to the use of nitrogen as an alternative carrier gas to helium. In this work we will determine if the same benefits are achievable for SVOCs on a new generation of mass spectrometer with reduced electric consumption and heat output.

A previously developed helium carrier gas based SVOC method was adapted for the use of nitrogen carrier gas by scaling the column dimensions to achieve equivalent chromatographic separation and runtime. Following method development on the latest model tandem quadrupole the dry source method was transferred to a previous generation tandem quadrupole. That system was operated with helium carrier gas and argon CID gas using the same MRM transitions as the all nitrogen system in order to generate comparative data.

Both the all-nitrogen system and the one using helium carrier achieved equivalent chromatographic separations and sensitivity. The equivalency of the data across the two configurations demonstrates the feasibility of both for performing quantification of multi-class SVOCs extracted from environmental matrices at sensitivity levels compatible with the scaling of sample preparation to reduce solvent consumption while also using a split injection to reduce matrix loading on the column.

#### **3.04.P-Th207 A Water Quality Stocktake: Identification of Priority Emerging Pollutants in Waters of England and Northern Ireland**

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An investigation was undertaken for the Office for Environmental Protection (OEP) with the aim of bringing together the latest scientific understanding on chemicals in the water environment in England and Northern Ireland. The primary focus was on 'emerging pollutants' and 'pollutants with significant new insights' because of the need for early identification of chemicals of concern, so that effective intervention can be undertaken prior to damage being caused to the environment, wildlife or human health. This review considered broad use categories for chemicals that included biocides, pharmaceuticals, industrial chemicals, personal care products, veterinary medicines, and endocrine disrupting chemicals.

A stepped approach was followed to identify relevant pollutants of greatest concern. An initial literature review identified 194 chemicals of most interest, which were then narrowed down to a shortlist of 74 classed as 'emerging' or with 'significant new insights'. Many of the substances could be grouped together, resulting in a total of 25 pollutants/categories of interest (9 individual substances and 16 groups) that were the subject of factsheets that compiled information on their primary sources, ecological & human health impacts and relevant legislation. The aim of the factsheets was to provide an accessible and objective summary of the key pollutants and support prioritisation for further work.

Focused risk assessment was undertaken for an initial set of 7 priority substances selected to ensure that there was a representative for each use category and on the basis of specific concerns highlighted in the factsheets. Risk assessment focused on the spatial extent of pollution as indicated by mapping of monitoring data, the magnitude of risk currently posed to the natural environment and human health & how this may evolve over time. Of the pollutants considered 1,4-dioxane (an industrial solvent) and fipronil (an insecticide used in flea treatments for pets) were considered to present the highest risk to the aquatic environment. Galaxolide (fragrance used in household products) and bisphenol A (utilised in plastics and polymers) were also found to pose a relatively a high risk. The pharmaceutical diclofenac appeared to present a high risk in England, but a low risk in Northern Ireland. Climbazole (a fungicide used in personal care products) and the pharmaceutical substance carbamazepine present a moderate level of risk.

#### **3.04.P-Th208 Environmental Assessment of Tire-Road Wear Particles and Tire Rubber Additives in Water and Biota Samples from Llobregat River Delta**

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Tire-Road Wear Particles (TRWPs) originate from the abrasion caused by the interaction between vehicle tires and road surfaces. Recent studies highlighted the significant role of TRWPs in contamination of environmental compartments such as soil, water, air and, biota. These particles, which include synthetic rubber, fillers, softeners, vulcanizing agents, and additives, pose ecological and health concerns. Specifically, Tire Rubber Additives (TRAs) and their Transformation Products (TPs) have raised interest due to their possible toxicity. The compound N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), finds wide use in tire manufacturing and research proved the lethal effects of his TP, 6PPD-quinone on coho salmon.

Other common TRAs include Hexamethoxymethyl melamine (HMMM), 1,3-diphenyl guanidine (DPG), 1,3-dicyclohexylurea (DCU), N,N-dicyclohexylmethylamine (M-DCA), and benzothiazole (BT).

This study explores the presence of TRAs and TPs in the Llobregat River Delta (Catalonia, Spain), an area comprising the second-biggest Spanish airport surrounded by urban, agricultural, industrial, and natural protected spaces. Water and biota (fish and macroinvertebrates) samples were collected in a sampling campaign covering 6 different sites. A preliminary analysis focuses on the quantification of six common TRAs and 6PPD-quinone. Sample preparation for water (n=14) required solid phase extraction (SPE) with Oasis HLB, whereas a more complex protein and lipid clean-up was implemented for biota samples (n=21). Different extraction solvents and SPE cartridges have been evaluated and compared based on analyte recovery and matrix effect. The instrumental analysis was performed by Liquid Chromatography coupled to High-Resolution Mass Spectrometry (LC-HRMS).

To date, the potential environmental and health impacts of TRWPs are unknown, mainly due to the lack of reliable data, the limited number of samples studied, the diversity of compounds, and the methodological challenges. The application of this method, and its extension to a larger number of compounds can help monitor the environmental fate of tire rubber additives and risk assessment.

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### **3.04.P-Th209 A Novel approach in addressing the challenges of monitoring multi-classes of POPs in a single run by GC-Ion Mobility-HRMS**

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Polychlorinated dioxins and furans are bio-accumulative molecules largely formed during combustion and industrial manufacturing processes. Their analysis is complex due to low level regulatory exposure limits and difficult sample matrices. Both are considered as persistent organic pollutant (POP) and could be widely found in environmental samples. Principal consequences derived from dioxins and furans exposure, even at low concentrations, are cancer, reproduction and growth issues, immune system diseases and endocrine effects. This is similar for another POP family, the polychlorinated biphenyls. It consists of 209 congeners, 12 of them known as "Dioxin-like PCBs". Applications for these compounds are heat transfer fluids, dielectric components and coolant fluids for electrical components. Even though their manufacturing was drastically decreased in the 1960's due to the proved toxicity, their chemical stability and lipophilicity cause some congeners to still be found today in environmental samples, like soils and sediments. Most reported health effects are dermal and ocular lesions, lowered immune responses, mutagenic effects, breast cancer and poor cognitive development in and motor control problems.

These species are mainly analyzed by high-resolution sector field mass spectrometry. We propose here a novel workflow involving ion mobility as an orthogonal criterion for identification and quantification, coupled to a high-resolution QTOF mass spectrometer. The benefit is a high flexibility for analyzing various classes of compounds in the same GC run with high sensitivity.

1  $\mu$ L samples were separated by GC (35 min run time, Restek 60 m 0.25 mm id 0.25  $\mu$ m column, HxCDD and HxCDF separation). A GC-APCI source was coupled to the timsTOF Pro 2 (Bruker) which enables fast and sensitive analysis of Dioxins, Furans and PCBs in the very same run. Criteria for validation and quantification of compounds were high mass accuracy, retention time, isotope pattern matching, MS/MS qualifiers and collision cross sections (CCS) from ion mobility filtering. Kendrick mass defect plots were applied for the extraction of the specific area of compounds containing Cl or Br from the complete GC/MS chromatogram.

All PCBs have been detected at an LOD of  $\leq$ 10-20 ppt. The dioxins were detected down to levels of 25-125 ppt, depending on the individual compound. Examples of the analysis of real-life samples like rapeseed oil, milk fat, sludge extract and ash are presented.

### **3.04.P-Th210 Analysis of rubber-derived contaminants in surface water by liquid chromatography coupled with a hybrid linear ion-trap-Orbitrap high-resolution mass spectrometry.**

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Rubber-derived organic contaminants are of emerging environmental concern because of their potential risks to aquatic organisms. For example, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q), which is a transformation product of the antioxidant 6PPD used in tires, has been identified to induce acute mortality in spawning coho salmon. However, the occurrence and fate of these contaminants in the aquatic environment are largely unknown, in part due to the

limited analytical methods available. Therefore, the objective of this study is to develop a method to analyze five PPD-Qs and four amine and urea-type rubber additives in the dissolved phase and suspended particulate matter of riverine and estuarine surface waters. The developed method extracts the dissolved phase using solid phase extraction (SPE) by hydrophilic-lipophilic balance cartridges. The suspended particulate matter was extracted using ultrasonic-assisted extraction followed by SPE cleanup. The separation and quantification were achieved by reversed-phase C8 chromatography coupled with positive electrospray ionization linear ion-trap-Orbitrap high-resolution mass spectrometry. Linearity for all compounds throughout the 12-points calibration range was >0.99. The instrument detection limit was from 0.098 to 12.5 ng/mL. The absolute recovery range was 5 to 90 % for HPLC water, 2 to 85% for environmental water, and 22 to 99% for suspended particulate matter. The surface water samples collected from the Rimouski River and the St. Lawrence River and Estuary (Canada) will be used to determine the limits of detection and quantification and validate the method. To our knowledge, this is the first research to use a hybrid linear ion-trap-Orbitrap mass spectrometry to analyze the four amine and urea-based rubber additives and five PPD-Qs in the dissolved phase as well as the suspended particulate matter of riverine and estuarine surface waters. The developed method will contribute to a better understanding of the occurrence and partitioning of these contaminants in water, which are key environmental processes affecting exposure risks.

#### **3.04.P-Th211 Monitoring and Risk Assessment of Contaminants of Emerging Concern in the Middle Tagus River Basin (Central Spain)**

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The Tagus River and its tributaries in Central Spain are crucial for over six million inhabitants (more than five million of them concentrated in the Madrid area). Unfortunately, overpopulation and related wastewater treatment plants, many different industries, and agricultural and livestock activities are also concentrated in the central area where tributaries from the Madrid region discharge. In this context, the environmental risk by contaminants of emerging concern (CECs) is very serious and the information about the pollution state of the river is very scarce. Therefore, the aim of this project has been the monitoring of the pollution state and risk assessment in the Tagus River before and after the Jarama River confluence (Madrid city input) in a downstream cross section of one hundred kilometers.

Waters were sampled from 19 sites, specially selected considering the presence of livestock and agricultural farms, pharmaceutical industries, and wastewater treatment plants. Six sampling campaigns were carried out seasonally along 2022 and 2023. To have a general picture of the area, the samples were analyzed for more than 50 parameters, including those of physicochemical nature, and those reporting the pollution caused by both metals and organic matter. However, the focus was the study of CECs (primarily pharmaceuticals). To undertake this study, a sensitive and selective liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method has been developed for the determination of over CECs. The separation was performed by using a reversed phase C18 column Zorbax Eclipse Plus RRHD (2.1 mm × 100 mm, 1.8 μm). Mobile phase was water and acetonitrile both them with 0.1% of formic acid. The mass spectrometer was a TSQ Quantiva triple quadrupole analyzer (QqQ) (Thermo Scientific, USA) operating in selected reaction monitoring. To isolate the analytes from the sample, a solid phase extraction (SPE) using the HLB sorbent was employed.

Preliminary results showed a serious degradation state in this area. The pharmaceutical industries and the highly populated areas cause a significant input of CECs, being a hotspot for antibiotics and antimicrobial resistance (AMR). In general, the concentration of those compounds decreases downstream, but not for all the CECs. A huge number of samples and analytes are still under data treatment, and more focused and detailed conclusions are expected.

#### **3.04.P-Th212 Development of an Analytical Method based on micro-Matrix Solid Phase Dispersion Combined with Gas Chromatography -Mass Spectrometry for the Determination of Bisphenols in Mussel Samples**

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Bisphenols (BPs) are widely used in manufacture of different products as paints, adhesives and plastics. They are added to plastics during production in order to improve their properties for industrial and domestic applications. As BPs are weakly bound to the polymer matrix, are susceptible to migration from plastics to marine environment. In environmental samples, BPA and its analogues have been found in marine water, sediment and biota in high concentrations.

The main aim of this paper is to develop a cheap, easy and fast method based on a miniaturized matrix solid phase dispersion (*micro*-MSPD) combined with GC-MS (mode SIM) by using a factorial design which is capable of determination of nine BPs in mussel samples. The bisphenols analysed are BPAF (2,2-Bis (4-hydroxyphenyl) hexafluoropropane), BPF (bis (4-hydroxyphenyl) methane), BPE (4,4'-ethylidenebisphenol), BPA (2,2-bis (4-hydroxyphenyl) propane), BPG (2,2-Bis(4-hydroxy-3-isopropylphenyl) propan), BPC2 (1,1-dichloro-2,2-bis(4-hydroxyphenyl) ethylene), BPZ (1,1-Bis(4-hydroxyphenyl) cyclohexane), BPS (4,4'-sulfonyldiphenol) and BPM (4,4'-(1,3-phenylenediisopropylidene) bisphenol). The sample preparation was made using Agilent Captiva EMR-lipid as dispersant and cleanup agent. To increase volatility,

enhance chemical and thermal stability and reduce polarity of analytes, derivatization with 99% N,O-Bis-(trimethylsilyl)trifluoroacetamide+1% trimethylchlorosilane (BSTFA+1% TMCS) was necessary. Validation has been successfully achieved for linearity, LOQ, recovery and precision. The validated method has been applied to five mussel samples coming from Galician Rías.

This method has several advantages when compared with other conventional extraction techniques as it is less time consuming and cheaper and it uses less solvent volumes.

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### **3.04.P-Th213 Multicomponent method for the ultra-sensitive quantification of non-polar pesticides in Swiss rivers and effluents**

*Vera Ganz*<sup>1</sup>, *Kim Luong*<sup>2</sup> and *Heinz Singer*<sup>1</sup>, (1)Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland, (2)Environmental Chemistry, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland Studies have shown that pyrethroids pose a major risk to aquatic organisms in small and medium-sized watercourses in Switzerland. These substances are toxic even in the low pg/L range. They tend to sorb to particles and accumulate in non-target organisms due to their high hydrophobicity and are the major contributors to ecotoxicological risk. However, detailed information on the occurrence, sources and input pathways of pyrethroids and other non-polar pesticides is missing. A sensitive multi-method is essential to investigate their occurrence in selected wastewater treatment plants and small watercourses, enabling the quantification of both dissolved and particle-bound concentrations. Obtaining the total concentration is indispensable for a proper risk analysis of non-polar compounds.

For this purpose, an existing liquid-liquid extraction (LLE) followed by gas chromatography atmospheric pressure chemical ionization (GC-APCI) coupled to a triple quadrupole (QqQ) mass spectrometer (MS) method for pyrethroids and organophosphate insecticides was further developed in order to facilitate the quantification of non-polar pesticides in the dissolved and particle-bound fraction (Rösch et al. 2018).

The compound list was expanded by about 70 PPPs and biocides with logP>4, notably, carbamates, diazines, coumarin rodenticides, etc. along with additional isotopically labelled internal standards. To quantify the widest possible range of substances, the extract was split and analyzed using complementary chromatography and ionization methods, namely GC-APCI and liquid chromatography electrospray ionization (LC-ESI), both coupled to a QqQ-MS. Hexane and cyclohexane as well as a combination of both, were tested as extraction solvents. Absolute recoveries were determined for each substance across the individual steps of the sample preparation, including the extraction from the water phase, addition of Na<sub>2</sub>SO<sub>4</sub> to dry the extract, evaporation by nitrogen and the dissolution. The limits of quantification (LOQs) for 60% of the compounds in the final method range from 10 to 500 pg/L, 37% have LOQs even under 100 pg/L.

The performance of the developed method will be demonstrated through the initial findings obtained from wastewater and river samples collected during a field campaign in Switzerland.

Rösch, et al., *Anal Bioanal Chem*, **2019**, doi.org/10.1007/s00216-019-01787-1

### **3.04.P-Th214 DIRECT AQUEOUS ANALYSIS OF PESTICIDES AND PPCPS IN DRINKING AND BOTTLED WATER AT PARTS PER TRILLION LEVELS**

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Drinking water analysis is subjected to extremely low and rigid prescribed consent values (PCV) for determinands. Water suppliers and utility companies need to ensure that the final water product they send out for bottling or into supply networks is safe and complies with state and country regulations. Water can be a challenging matrix, in particular if it is sourced from a river, lake or reservoir before entering the water treatment process.

Here, a series of experiments was conducted in collaboration with Vitens Water Company, to test several types of water samples spiked with a mixed standard of 431 determinands, including pesticides, pharmaceuticals and personal care products.

Direct aqueous injection was performed of spiked water samples - MilliQ water, tap water, Evian bottled water and Fiji bottled water. The samples were spiked with a custom mix standard, containing 431 analytes. A series of 11 standards were made in the respective matrices at sequential concentrations from 0.1 to 500 ng/L. A collection of unknown blank samples was also included in this study. The analysis was performed using a SCIEX 7500 system, operated in both positive and negative electrospray ionization with fast polarity switching.

The analytical method presented shows ultra-high levels of sensitivity with LOD values of 0.1 ng/L reported. All data was acquired through direct aqueous injection with no SPE needed. Good chromatographic peak-to-peak separation was achieved with a total run time of 25 minutes. Even at low level concentrations, excellent levels of precision and accuracy were achieved (e.g. %CV  $\leq$  6.76 at 0.1 ng/L). There is scope to expand the number of analytes tested and apply this system to other individual workflows which demand very low limits of quantification. This method provides solutions for drinking water analysis and problematic determinands such as dicamba.

### **3.04.P-Th215 Exploring the impacts of primary and secondary aerosols from emerging sources on human health**

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Atmospheric fine aerosols was the fifth mortality risk factor in 2015, causing 7 million deaths and 203.1 million disability adjusted life years (DALY). Sources of air pollution in the indoor and outdoor environment include vehicle emissions, biomass burning, cleaning products, and many other anthropogenic and natural sources. More recently, the emissions from the usage of volatile chemical products, such as pesticides, coatings, flame-retardants, adhesives, cleaning agents, and personal care products are rising. Despite some success in mitigating air pollution in certain countries, 90% of the world's population still live in areas exceeding the guidelines of the World Health Organisation. The hazard quantification of the human exposure to atmospheric aerosols is largely derived based on epidemiological studies however, to date, little is known for the mechanisms that underpin the exposure and can induce biological responses.

Recently, in-vitro toxicological studies have revealed the inflammatory responses of cell lines from the exposure to aerosol particles from a number of sources. However, the majority of the studies on VCPs are focused on the quantification of primary emissions from indoor or outdoor sources. An increasing number of studies have showed that VCPs can react efficiently with atmospheric oxidants, such as the hydroxyl radicals, leading to the formation of a plethora of currently unknown secondary species, known as secondary organic aerosols (SOA). Exposure to SOA has been strongly associated with cardiorespiratory disease mortality, while laboratory studies have showed that SOA exhibit increased toxicity with atmospheric transformation processes. Despite this evidence, the environmental persistence and health impacts of the VCP-SOA remain unknown.

This work aims to provide novel chemical and biological descriptions of the atmospheric behaviour and impacts of pesticides as representative VCPs. By developing novel experimental multidisciplinary techniques, this work investigates the atmospheric fate, the SOA formation potential, and physicochemical characteristics of selected pesticides atmospherically relevant conditions. Further, standardised sampling protocols have been developed to demonstrate the capability for accurate biological assessment of their health hazards. This will be the first study of its kind to systematically investigate such complex systems.

### **3.04.P-Th216 Towards Standardization of the Biomimetic Extraction using Solid-Phase Microextraction (BE-SPME) Analytical Method**

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Biomimetic extraction using solid-phase microextraction (BE-SPME) is a passive sampling analytical method that can predict the aquatic toxicity of complex petroleum substances. The method provides a non-animal biomonitoring alternative to traditional bioassays that can reduce both vertebrate and invertebrate aquatic toxicity testing. The technique uses commercially available polydimethylsiloxane-coated fibers that, following non-depletive extraction of water samples, are injected into a gas chromatograph with flame ionization detection. During BE-SPME analysis, the solid-phase microextraction step can be conducted either manually or using an autosampler. Recent round robin research program comparing results from 10 laboratories reported that the results from the automated BE-SPME method yielded much lower inter- and intra-laboratory variability relative to the results obtained using the manual method. While the source of the variability in results from the round robin was suspected to be caused by the mixing rate and type during the SPME fiber-sample equilibration, additional data from analyses of duplicate water samples at multiple laboratories using only the automated method showed significant interlaboratory variability (50-100% relative percent difference). This high interlaboratory variability hampers comparisons across studies and pooling of data to allow reproducible hazard assessments of waters containing complex petroleum substances. Additional research involving multiple laboratories has been initiated to identify other potential sources of interlaboratory variability of BE-SPME with the aim of standardizing the method. This presentation will discuss the main causes of identified interlaboratory variability and potential solutions.

### 3.05 Analytical Developments and Challenges in Detection and Monitoring of the Growing Universe of Per- And Polyfluoroalkyl Substances (PFAS)

#### 3.05.T-01 Unravelling the Combination of Atmospheric Pressure Ionization Sources to Extend the PFAS Coverage

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Per- and polyfluoroalkyl substances (PFAS) involves a large group of compounds (more than 6,300) covering a vast variety of chemical structures going from ionic to neutral PFAS that are classified as contaminants of emerging concern. Although they are usually analyzed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) using electrospray ionization (ESI) for both targeted and non-targeted screening (NTS) approaches, the upgrade of analytical strategies to fulfill the monitoring requirements for a wide-scope determination of PFAS, especially PFAS with low/null ionization efficiency by ESI is still limited. Thus, this work aims to propose high-throughput cutting-edge methodologies based on HRMS combining atmospheric pressure ionization sources such as ESI and atmospheric pressure chemical ionization (APCI) to sort out the complexity on the analysis of PFAS by extending the analyte coverage of the LC-HRMS methodologies.

Although ESI provides an efficient ionization for most of ionic PFAS, the ionization of less polar fluorotelomers (FTs) is less favored due to their higher difficulty to generate the  $[M-H]^-$ . Thus, it was observed that ESI provided slightly better instrumental limits of detection (iLODs) around 16-fold lower than those achieved by APCI for ionic PFAS, while for FTs, APCI showed iLODs up to 3-fold lower than ESI. This is specially relevant for low-polar PFAS such as fluorotelomer alcohols that were only ionized by APCI through characteristic series of in-source fragment ions differing in 20 Da (HF losses). Using this approach, the use of orthogonal ion sources not only allows to extend the range of PFAS but also helps to increase the confidence on the annotations ionized with both sources. Besides, this approach allows the establishment of new NTS strategies using either the observed ion/s or ESI/APCI correlations under fix conditions. Thus, the neutral losses of HF due to the in-source fragmentation observed for FT-based PFAS by APCI were used as data mining strategy to prioritize features. This was easily visualized in Kendrick mass defect plots (HF scale) which group series of in-source fragment ions belonging to the same FT compound. Finally, a model for the estimation of the concentration of tentatively identified PFAS (when no standard is available) was set based on the normalized relation between ESI and APCI responses showing the great potential of this analytical approach to dive deeper in the NTS of PFAS.

#### 3.05.T-02 Quantitative Assessment of Poly- and Perfluoroalkyl Substances (PFASs) in Aqueous Film Forming Foam (AFFF) Impacted Soils: A Comparison of Analytical Methodologies

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Poly- and perfluoroalkyl substances (PFASs), particularly perfluoroalkyl acids (PFAAs) like perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), have received significant attention due to their widespread contamination, environmental persistence, bioaccumulation, and toxicity. Aqueous film forming foams (AFFFs) are an important source of PFAS releases to both groundwaters and soils. While AFFF formulations are known to contain fluorinated surfactants, their exact compositions are proprietary and manufacturer-dependent. The diversity of PFAS chemistries at AFFF-impacted sites is not only due to fluorochemical manufacturing processes, but also the fact that the wide-range of polyfluoroalkyl chemistries used in AFFF can transform into an even wider range of PFASs upon release into the environment, particularly in soils. Many of these PFASs are likely significant sources of PFAAs due to *in situ* transformations. Accurately quantifying the full suite of PFASs at AFFF-impacted sites, particularly in soils, is essential in understanding the potential risk an AFFF impacted site poses to surrounding humans and biota. The objective of this study was to assess the performance of various analytical approaches for extracting and quantifying  $\Sigma$ PFASs in AFFF-impacted soils, with a particular focus on analytical approaches that enable both liquid chromatography high resolution mass spectrometry (LC-HRMS) analyses and total organic fluorine measurements in the same extract. Draft EPA Method 1633 was evaluated, as were modifications to 1633 to ensure that a comprehensive list of anionic, cations, and zwitterionic PFASs could be analyzed for AFFF-impacted sites. Three AFFF impacted soil composites were analyzed using the modified 1633 methodology, 1633 as currently proposed, and a previously published comprehensive extraction and cleanup method. Targeted and suspect screening analyses were performed in both electrospray negative (ESI-) and positive (ESI+) modes. In addition, spike experiments using clean matrices, native PFASs, and mass labeled PFASs were conducted to determine absolute compound recoveries of the three methodologies. The results suggest that with minimal modifications, this modified 1633-like methodology is capable of quantifying a wide range of PFASs in AFFF-impacted soils while maintaining compatibility with total organic fluorine-type analyses.

#### 3.05.T-03 Analysis of Per- and Polyfluoroalkyl Substances (PFASs) in Consumer Food Packaging by Different Analytical Approaches

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Diet is considered one of the major routes of human exposure to PFASs which can be present in both the food itself but also the food contact materials (FCM) and migrate into food. In this study, we tested 88 consumer food packaging samples from different geographical locations for PFASs. Thousands of PFAS chemicals are used in commerce, however, information on their identity and use is lacking, plus limited amount of PFAS analytical standards are commercially available for method development and routine testing. To address these challenges and to expand the analytical scope, this study utilized various sample preparation and instrumental analysis approaches. Sample extraction and controlled migration experiments were utilized to determine PFAS occurrence and levels in food packaging samples. In addition, total oxidizable precursor (TOP) assay was utilized to capture PFAS precursors. Both targeted and non-targeted analyses using liquid chromatography tandem mass spectrometry and Orbitrap high resolution mass spectrometry (HRMS) were employed. Targeted analysis for 34 PFASs resulted in 19 analytes detected in the tested samples after extraction. Overall, 84% of the samples had detectable levels of at least one PFAS, with 6:2 fluorotelomer phosphate diester (6:2 diPAP) found most frequently and at the highest levels (up to 224 ng/g). Other frequently detected substances were PFHxS, PFHpA and PFDA. PFHpA, PFPeA and PFHxS were present at levels up to 51.3, 24.1 and 18.2 ng/g, respectively. The TOP assay results revealed that average  $\Sigma$ PFAS levels increased over 10 times after oxidation, suggesting the presence of undetected PFAS precursors. Four PFAS were found migrating into food simulants during controlled migration tests: PFHxS, PFHpA, 6:2 diPAP and PFHxA. Based on the highest migrated measured levels, weekly intake values were calculated and were below the established EFSA maximum total weekly intake of 4.4 ng/kg body weight/week. Finally, non-targeted analysis was utilized to discover and identify PFAS not covered by the limited scope of our targeted approach. Multiple HRMS workflows were developed and validated utilizing signatures unique to PFAS compounds. Results from the non-targeted analysis of food packaging samples showed 41 potential PFAS features with 8 being found in all four food and material types. Five tentatively identified compounds were confirmed with analytical standards, and quantified using retrospective data analysis.

### **3.05.T-04 Characterization of Fluoropolymers for Residual PFAS: Implications for Munitions, Thermal Degradation, and Human Exposure**

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To dispose of unserviceable explosives and munitions, open burns are conducted by the United States Department of Defense as part of demilitarization programs. However, there is growing concern over possible air emissions and associated risk to humans and the environment produced during open burns of munitions containing fluoropolymers. While fluoropolymers themselves do not appear to pose a significant risk, there is evidence that at high temperatures fluoropolymers thermally degrade to non-polymeric PFAS of toxicological significance as well as hydrofluorocarbons, a class of greenhouse gases. Additional fluorinated compounds are found as residuals used in the synthesis of fluoropolymers. Prior to thermal degradation, a thorough characterization of pure and cross linked fluoropolymers is needed to understand the range of gas-phase thermal degradation byproducts that can potentially form. The total fluorine content of pure and cross-linked fluoropolymers was determined using instrumental neutron activation analysis and characterized by pyrolysis gas chromatography mass spectrometry (GC-MS). A simple method was developed to extract fluorinated residuals from fluoropolymers. A novel, in-tube <sup>19</sup>F and <sup>1</sup>H nuclear magnetic resonance spectroscopy (NMR) method was then used to identify the organic solvent most effective for extracting fluorinated residuals. Extracts also underwent analysis by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF) for target, suspect, and non-target analysis via Kendrick mass defect for nonvolatile PFAS and by GC-MS for target volatile PFAS. Thermogravimetric analysis with an evolved gas analyzer was performed to understand the range and quantity of thermal degradation products that form when fluoropolymers and their fluorinated residual thermally degrade. Acetone was selected as the most effective solvent for swelling and extracting residuals. Individual PFAS contributed to <1% of total fluorine in fluoropolymers. By LC-QTOF, polytetrafluoroethylene consisted of 0.43 ng/g to 2.8 ng/g of a series of C4 to C16 perfluoroalkyl carboxylic acids (PFCAs). Additionally, NMR was used to identify the presence of bisphenol AF and non-targeted unknown PFAS in crosslinked Viton A. With the presence of PFCAs, during thermal degradation there exists the potential for fluoroethenes to form, which are neurotoxins, that creates an exposure risk for those conducting open burns.

### **3.05.P Analytical Developments and Challenges in Detection and Monitoring of the Growing Universe of Per- And Polyfluoroalkyl Substances (PFAS)**

#### **3.05.P-Th217 Quantification of ultrashort per- and polyfluoroalkyl substances (PFAS) in water samples via headspace gas chromatography-mass spectrometry (GC-MS) - a method development**

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Per- and polyfluoroalkyl substances (PFAS) were classified as a group of highly concerning chemicals over the last decades. Because of the high persistence of PFAS, their previous use led to contamination of the environment and human population. Due to ongoing use and incomplete remediation or destruction methods, the PFAS background in the environment is growing continuously.



The presence of ultrashort PFAS (with  $\leq 3$  carbon atoms) in the environment is often overlooked despite their contributions to PFAS levels. These compounds may directly contaminate the environment and arise from degradation of polyfluorinated compounds as well as incomplete PFAS destruction in certain industrial processes. Nonetheless, few approaches for targeted analysis of ultrashort PFASs have been developed, except for trifluoroacetic acid (TFA). Beside liquid (LC-MS/MS), supercritical fluid (SFC-MS/MS), and ion chromatography (IC-MS)-based systems, gas chromatography coupled with mass spectrometry (GC-MS) is a promising method for detection and quantification of ultrashort PFAS.

The goal of this study was to develop a simple headspace GC-MS method for the quantification of ultrashort perfluorocarboxylic acids (PFCAs) and polyfluorinated alcohols (PFOHs) in water samples. In contrast to PFOHs, functionalization of PFCAs was required for quantification. This was done by esterification with methanol at 80 °C, which can be carried out directly in the headspace GC-MS system.

Moreover, several parameters were optimized to achieve a low limit of quantification for the analytes used: i) The ratio of the aqueous solution, methanol, and available gas phase within the analysis vessel, ii) the concentration of additional acid in esterification mixtures of PFCAs, iii) shaking frequency and iv) shaking time before analysis. After optimizing the procedure, we were able to quantify ultrashort PFCAs and PFOHs. Thus, our developed headspace GC-MS method has the potential to be used as an alternative target analysis for ultrashort-chain PFCAs and PFOHs in various water samples (groundwater, wastewater).

### 3.05.P-Th218 Per- and Polyfluoroalkyl Substances (PFAS) Precursors in Dutch Surface Waters

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PFAS are a huge group of chemicals with unique properties used in aqueous film-forming-foams (AFFFs) or in manufactured products such as fabrics or food packaging materials. However, after their use, they end up in the environment and can contaminate water, soil and air. Surface waters are important to monitor because humans and wildlife depend on them to live. Legacy PFAS like for example PFOS (perfluorooctanesulfonic acid) or PFOA (perfluorooctanoic acid) are usually monitored because they are bioaccumulative and negatively affect human health. PFAS precursors can be an important source of contamination because they can be degraded into parent compounds which accumulate in the environment or the human body. This work focuses on the identification of PFAS precursors and legacy PFAS in Dutch surface water samples using reference standards synthesized beforehand. Synthesized compounds include *N*-sulfo propyl perfluorohexane sulfonamide, short-chain perfluoroalkane sulfonamide derivatives (C4 and C6) namely *N*-alkyl perfluoroalkane sulfonamide and *N*-alkyl perfluoroalkane sulfonamidoacetic acid, *N*-trimethylammonio propyl perfluoroalkane sulfonamide (C4, C6 and C8), 6:2 fluorotelomer sulfonamide, 6:2 fluorotelomerthia propanoic acid, *N*-dimethyl ammonio propyl perfluoroalkane sulfonamide (C4, C6 and C8) and *N*-sulfo propyl dimethylammonio propyl perfluorohexane sulfonamido propyl sulfonate. Twenty surface water samples from the Netherlands were extracted by solid phase extraction (SPE) then analysed by liquid chromatography – tandem mass spectrometry (LC-MS/MS). The sum of the analysed PFAS was on average 25-30 ng/L, for every sample except three that showed a concentration higher than 60 ng/L. PFOS, PFOA, PFBS, PFBA, PFHxA and PFHxS were the legacy PFAS with the highest concentrations (5-17 ng/L). Targeted precursors were detected in almost all samples, especially *N*-methyl perfluorobutane sulfonamidoacetic acid (MeFBASA) (around 0.2 ng/L) and SPr-FHxSA. A source of SPr-FHxSA might be the use of AFFFs since this compound was mainly identified in these foams. MeFBASA was five times higher (1.1 ng/L) in one sample close to Tilburg. The reason is still unknown but it may be related to the textile industrial past in this region. Other samples will be analysed in this region to follow up this elevated concentration.

### 3.05.P-Th219 PFAS in Sub-Antarctic Seabirds: Evidence for Long-Range Transport and Bioaccumulation of Emerging Contaminants

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Poly- and perfluoroalkyl substances (PFAS) are a large group of chemicals that are characterised by a hydrophilic moiety (e.g., SO<sub>3</sub><sup>-</sup> or COO<sup>-</sup>) and a hydrophobic perfluorocarbon chain that varies in length. PFAS display unique properties due to combined oil and water repellence, extreme stability, and hence are used in a wide range of industrial processes and consumer products. These chemicals can be problematic in the environment due to their potential to undergo long-range transport, persistence and toxicity. Some longer-chain PFAS have already been listed in the Annexes of the Stockholm Convention and included in other international regulations, but replacements have started to emerge. Furthermore, the European Chemicals Agency (ECHA) is seeking to ban the use and production of PFAS (defined as chemicals containing CF<sub>2</sub>) completely in Europe. As such, it is important to document current levels of both legacy and emerging PFAS in the environment prior to this proposed ban so that any resulting decline in concentrations can be assessed accurately. Here, we employ an ultra-performance liquid chromatography coupled to a high-resolution mass spectrometer (Orbitrap) to analyse a range of both legacy and emerging PFAS including 11 PFCAs (C4-C14), 9 PFSAs (C4-C10), 3 FASAs, 3 PFPIAs and 6 fluorotelomer acids, among others. We have analysed liver tissues of black-browed albatrosses, white-chinned petrels and common diving petrels collected from the Falkland Islands and South Georgia from the early 2000s to the mid-2010s. The data show varying spatial and temporal trends between different species and but similar profiles for individual compounds; we draw comparisons between trophic levels by employing stable isotope analysis. Trends in these data reveal various pathways e.g., atmospheric and hydrospheric, for PFAS

into the Southern Ocean before bioaccumulating through food chains. Our research addresses the need to understand exposure levels in wildlife to PFAS compounds in areas close to and remote from sources.

### **3.05.P-Th220 Potential Impacts of COVID-19 on Per- and Polyfluoroalkyl Substances (PFASs) in Air in the Great Lakes Basin**

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Under Canada's Great Lakes Basin (GLB) Monitoring and Surveillance Program per- and polyfluoroalkyl substances (PFASs), are being monitored in air at two regionally representative background stations, namely Point Petre located on the north shore of Lake Ontario and Evansville located on Lake Huron. At Point Petre, air samples were collected from October 2018 to December 2022. At Evansville, samples were collected from May 2019 to December 2022. Air samples were taken once every 36 days using a high volume air sampler with a glass fiber filter (GFF) and a polyurethane foam-XAD sandwich (PUF/XAD/PUF). Air samples were analyzed for 19 ionic PFASs and 7 neutral PFASs. Most PFASs were detectable in greater than 50% of all air samples. Perfluorobutanoic acid (PFBA), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) were the dominant ionic PFASs while 6:2 and 8:2 fluorotelomer alcohol (FTOH) were the dominant neutral PFASs found in air. Generally higher air concentrations were found at Point Petre compared to Evansville as the Point Petre location is closer to urban centers, industrial activities and waste streams. During the COVID-19 pandemic, a significant decline in  $\sum$ PFASs concentrations in air was observed before and after 2020 reflecting the decrease in human and industrial activities in the region during COVID-shutdown. Multimedia environmental modelling revealed that the main in flow of PFOA and PFOS to Lake Ontario is via wastewater treatment plant effluents and tributary input; while water outflow from the lake, rather than sediment burial, is the main removal process.

### **3.05.P-Th221 Detection of Legacy and Emerging PFAS in Wastewater from a Fluoropolymer Production Facility in Italy**

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Studying the environment near manufacturing sites of per- and polyfluoroalkyl substances (PFAS) enables us to evaluate current, but also former PFAS releases and potential forthcoming PFAS concerns.

Employing suspect screening (SS) and non-targeted analysis (NTA) using high-resolution mass spectrometry (HRMS) has shown to be a suitable method for detecting a wide spectrum of PFAS, including new and potentially less-studied contaminants.

This study has been chosen as a case study for the European Partnership for the Assessment of Risks from Chemicals (PARC) and is being conducted in collaboration with the USEPA. Waste water samples of a fluoropolymer manufacturing site located in Northern Italy were analyzed by liquid chromatography coupled to HRMS (Orbitrap) and were processed with Compound Discoverer (Thermo Fisher). State-of-the-art methodologies designed for NTA of environmental samples, such as mass defect filtering, and identification of compound series by Kendrick plots, were employed to identify unknown or suspected PFAS.

The investigation showed the ongoing discharge of legacy PFAS (e.g. PFOA), despite their discontinuation in the fluoropolymer production a decade ago. Furthermore, alternative fluorosurfactants, fluorinated monomers and byproducts were identified. The findings show a chronological progression of fluorinated surfactants at this manufacturing site, i.e. a transition from C8-PFAS to short-chain PFAS, and ultimately to non-fluorosurfactant applications in the fluoropolymer production. However, the results also demonstrated the continued production or utilization of long-chain PFAS, despite increasing concerns regarding their bioaccumulative potential in aquatic and terrestrial food chains. Additionally, the investigation provides insight into environmental emissions resulting from the production of novel fluorinated polymers within the context of developing new materials for energy transition.

### **3.05.P-Th222 Comprehensive Screening for Per-and Polyfluoroalkyl Substances (PFAS) in Fish From More Than 100 Water Bodies in Southern Germany**

*Jessica Fischer, Bavarian Environment Agency (LfU), Augsburg, Germany*

This project explores the bioaccumulation potential of per-and polyfluoroalkyl substances (PFAS), a profoundly concerning group of substances, in fish. Analyses for up to 42 PFAS were conducted on pooled muscle samples (filet of 3 - 15 individuals) from more than 90 Bavarian inland water bodies, with focus on smaller rivers and streams. Sites were selected based on prior water analyses that triggered fishing activities preferably during the same year, when perfluorooctane sulfonate (PFOS) concentrations exceeded . This threshold, equivalent to three times the environmental quality standards (AA-EQS) for PFOS in inland surface water, was chosen anticipating no exceedance of the biota-EQS below this level.

Furthermore, larger rivers were sampled over three consecutive years to uncover potential short-term trends. The seven Bavarian trend monitoring sites under the water framework directive (WFD) were selected for these annual investigations, considering previous data and an abundant availability of fish of the same species (*Squalius cephalus* or *Rutilus rutilus*). These analyses extended beyond pooled muscle samples and included the filet of every individual captured per site (usually 15 - 25).

PFOS was detected in every sample and mostly as the predominating compound. Significant variation within individuals of the annually sampled sites, in some cases spanning an order of magnitude, Perfluorooctanoic acid (PFOA) was only found in fish of two nearby water bodies, linked to pre-2008 industrial use (fluoropolymer production). Other targeted perfluorocarboxylic (C4 - C18) and perfluorosulfonic acids (C4 - C13) were detected in a limited number of cases, with PFSA's being barely found, while PFCAs were more frequently identified. Among them, perfluorononanoic acid (PFNA) dominated in both number of occurrences and concentrations, followed by longer-chain compounds (C > 10). None of the targeted replacement chemicals, including components of F-53B (9Cl-, PF3ONS and 11Cl-PF3OUdS), DONA and Gen-X / HFPO-DA were detected. The same applied to precursors (10:2-, 8:2-, 6:2- and 4:2-FTSA, EtFOSA, N-EtFOSAA, N-EtFOSE, MeFOSA, N-MeFOSAA, N-MeFOSE, PFOSA) as well as to degradation products and other PFASs (H<sub>4</sub>PFUnA, H<sub>2</sub>PFDA, HPPHpA, PF-3,7-DMOA). This ongoing five-year project provides a unique opportunity to gain insights into bioaccumulation as it provides a vast set of fish and corresponding water data.

### 3.05.P-Th223 Environmental and multisource monitoring of PFAS in a pan-European perspective

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Establishing a process for environmental and multisource monitoring is a key component in the Horizon Europe Partnership for the Assessment of Risks from Chemicals (PARC), with the purpose of supporting the chemical risk assessment of the European Union (EU). A pilot project was launched in this task to develop and validate a workflow which could become the model for future monitoring campaigns in PARC. Per- and polyfluoroalkyl substances (PFAS) are one of the compound groups selected for this pilot project. It intends to establish a PFAS baseline for Europe to reflect the current environmental levels of PFAS resulting from decades of PFAS production and use. Using available monitoring data, PFAS baseline levels will be derived for different regions, matrices and land uses which can serve as a benchmark for future comparisons. Besides this baseline, a set of case studies will address PFAS pathways from source to aquatic recipients. The case studies will focus on European sites with a confirmed PFAS contamination (e.g., firefighting training sites, wastewater-impacted areas, fluoropolymer production facilities). Using a combination of advanced methodological approaches (target analysis, suspect and non-target screening, and sum parameters such as TOF and TOP assays), the case studies will determine the role of PFAS precursors in the environmental fate of PFAS (transport and transformation pathways), and explore the possibility of using PFAS fingerprinting to improve the understanding of PFAS pathways and source identification. Following the actual monitoring work, the pilot study will be concluded with an analysis of lessons learned and potential adjustments in future environmental monitoring projects under PARC.

### 3.05.P-Th224 Per- and Polyfluoroalkyl Substances (PFAS): Validation of Methodology for the Determination of Residues in Surface Water and Plasma

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Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic organofluorine compounds that have become a concern due to their persistence in the environment following extensive use over the last few decades. Due to their high degree of chemical stability, as well as their widespread use, PFAS chemicals now are increasingly being detected in the environment and appear to persist for extended periods of time. Furthermore, exposure to these chemicals in everyday objects, such as packaging and cookware, as well as within certain areas of industry, has led to the apparent bioaccumulation in certain areas of the population. Because of the concerns over health and environmental issues associated with PFAS chemicals, there has been a need to be able to monitor the occurrence of these materials in humans and the environment.

There are numerous approaches to analysing a range of these chemicals in a number of different matrix types, and this area of analytical chemistry is constantly evolving as new PFAS chemicals are identified and added to the list of those where methods already exist.

The work described in this poster was a project to develop and validate an LC-MS/MS method for the analysis of a "typical" suite of PFAS chemicals in the environment (surface water) as well as in body fluids (plasma). A simple and robust method was required, that could be easily applied to routine analysis at low concentrations, using commonly available equipment and approaches. There was also a requirement to be able to add additional PFAS analytes to expand the method as the need arises.

The resulting method of analysis of PFAS chemicals in plasma involves extraction/precipitation with methanol, followed by dilution with aqueous ammonium acetate. Surface water samples were prepared by the addition of ammonium acetate and methanol. Quantitation was performed by LC-MS/MS.

The method was successfully validated for 22 PFAS analytes in plasma and 21 in surface water at limits of quantitation (LOQ) of 10 ppb and 0.1 ppb respectively. Detection limits were equivalent to 2 ppb and 0.02 ppb for plasma and surface water. The validations were performed in accordance with the SANTE/2020/12830 guidelines to ensure that current and future performance requirements were met. Further work will include additional analytes to the PFASs already validated, as well as to investigate approaches to reduce the LOQ of the method to increase its sensitivity.

### **3.05.P-Th225 PFAS Reference Materials: From Industrial Precursors and Technical Grade to High Purity n-Isomers**

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Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals with widespread industrial and commercial applications. Their persistence and potential health effects raise significant environmental concerns. Accurate measurement of PFAS in environmental and biological samples is crucial for assessing exposure and risk. High-quality reference materials (RMs) and certified reference materials (CRMs) are essential for this purpose.

Since 1950, PFAS compounds have been widely used in various industrial and commercial applications. The global manufacturing has employed two processes to produce perfluoroalkyl chain compounds. Electrochemical fluorination produces a mixture of linear and branched isomers; telomerization typically yields an isometrically pure (or enriched) linear product. These techniques have resulted in PFAS with varying isomeric purities and also other organic impurities, water, solvent, and inorganic residues. These contaminants affect chromatographic and net purity, influencing analytical detector response and compromising the reliability of quantitative analysis. PFAS homologues, linear and branched PFAS isomers may exhibit distinct environmental and toxicological properties and metabolic profiles. Therefore, reference material producers must provide a range of fully characterized reference standards and labeled internal standards.

Numerous environmental samples contain both branched and linear isomers of PFASs. This mixture poses challenges in accurately quantifying many PFASs in environmental matrices, quantitative analysis of PFASs often involves eluting all isomers together and integrating them as a single peak. Isomer-specific PFAS analysis is hindered by limitations in analytical instrument development and methods. Additionally, the lack of high-quality, fully characterized analytical reference standards for isomer-specific identification and purity further impedes accurate PFAS analysis.

This study focuses on synthesizing, purifying, and characterizing isometrically pure PFAS reference materials, primarily n-isomers of different PFAS compounds. The development of PFAS reference materials from technical grade materials to pure isomer RM and CRMs will be addressed. The synthesis and purification process, and the differences in analytical approaches for bulk reference standard characterization and environmental analysis at trace levels will also be discussed.

### **3.05.P-Th226 Laboratory Considerations when Analysing PFAS Containing Samples**

*Matt James and Anthony Edge, R&D, Avantor Sciences, United Kingdom*

The LC-MS determination of PFAS in environmental matrices is challenging due to the increasingly low-level quantification required by regulatory authorities. This challenge is exacerbated by the use of PFAS containing materials within the laboratory environment, which can result in inadvertent contamination of analytical samples and invalidation of analytical data. To avoid quantification issues, it is essential to characterise potential sources of contamination and to apply rigorous working practices to eliminate them. This work summarises experiences from within our laboratory when establishing a PFAS LC-MS method within a working “non-PFAS” laboratory and is intended to serve as guidance for analysts new to the field of PFAS analysis.

Initially, common laboratory consumables used during sample preparation were screened by LC-MS to identify materials that could potentially contaminate samples. Several consumables were found to contain extractable PFAS. This data was used to eliminate/control sample handling and preparation steps that could potentially contaminate samples. The LC-MS system and consumables were also thoroughly assessed; background PFAS components (PFHxA, PFHpA, PFOA, PFNA and PFDA) were found to originate from LC-MS solvent line tubing and the use of a PFAS delay column was shown to resolve the problem. Additionally, the importance of testing consumables, including sample vials was demonstrated. In one case, a vial cap material recommended for PFAS analysis was found to leach 4,8-dioxa-3H-perfluorononanoic acid (ADONA) into methanolic diluent.

Many PFAS workflows utilise sample pre-concentration by solid phase extraction (SPE). This step was anticipated to be potentially problematic, indeed persistent contamination of PFHxA from the nitrogen blowdown step was found to originate from compressed air and nitrogen line tubing. Various tubing materials were assessed, with several found to contain extractable PFAS. An additional concern with sample preparation was the potential for high PFAS load water samples to cross-contaminate other samples when undergoing parallel or subsequent processing. A novel rapid pre-screening method was developed, which allows a small 200µL aliquot of sample to be screened in under two minutes using a 10 mm column and one

minute gradient. By applying this approach, laboratories may quickly pre-classify incoming samples and divert high PFAS samples to alternative work streams to safeguard against cross contamination.

### **3.05.P-Th227 Overcoming Solvent Effects at High Injection Volumes in PFAS Analysis**

*Marcus James Chadha, PhD, Agilent Technologies, Inc., United Kingdom*

The challenge of low PFAS detection and quantification limits: The analysis of PFAS (per- and polyfluorinated alkyl substances) often requires sample preparation techniques like solid phase extraction (SPE), especially in case of drinking water analysis with low detection limit requirements. The final samples ready for LC/MS/MS analysis are therefore usually dissolved in 80-100% organic solvents.[1] Additionally, recommended drinking water concentration limits are getting even lower, lately.[2] Injecting high sample volumes could improve sensitivity and therefore allow lower detection limits but this is limited by undesirable solvent effects caused by the high elution strength of the sample solvent in case of common reversed phase liquid chromatography.

Overcoming solvent effects in a convenient way: Here we present how the use of feed injection, as an alternative injection principle to the common flow through injection allows much higher injection volumes without negative impact on the peak shape, even when the sample is dissolved in 100% organic solvents. This is achieved by infusing the sample into the mobile phase stream with a special valve resulting in a dilution.[3] The use of a novel C18 reversed phase column designed to be compatible with a 100% aqueous mobile phase helps to maximize the improvement.

### **3.05.P-Th228 PFAS Analysis to Address the EU Regulations for 24 Compounds**

*Day Powell, Agilent Technologies, Inc.*

Poster to highlight the latest analytical techniques to test for PFAS (Per & polyfluoroalkyl substances) in various Matrices including Drinking water and Raw waters. Data and results to be included using the latest analytical techniques used to analyse for over 24 PFAS including the latest 24 PFAS in the EU regulations, including Liquid-Chromatography/Mass-Spectrometry and Gas-Chromatography/Mass-Spectrometry, with the latest developments in liquid-chromatography hybrid sampling techniques and online-Solid Phase Extraction. These developments have allowed for the analysis to overcome many challenges associated with PFAS analysis reaching lower levels of detection across wide ranges of challenging matrices and compounds. The new methodologies can also be applied to other PFAS compounds such as in the UK regulatory compounds, now containing 48 PFAS compounds, and allow analysts to reach the new parts per quadrillion levels set in the USA by the EPA for water analysis. New injection/preparation techniques described include online-Solid Phase extraction which allows the analysis of complex matrices such as foods or biological samples with less interferences and more reproducibility in results. This improved analysis will allow for accurate research and environmental modeling of PFAS.

### **3.05.P-Th229 Occurrence of Poly- and Perfluoroalkyl Substances (PFAS) in Transitional and Marine Water along the Dublin Coast**

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Poly- and perfluoroalkyl substances (PFAS) are a large group of compounds consisting of a partially or fully fluorinated carbon chain. Due to their physio-chemical properties these substances are widely used across a range of industries including electronic and pharmaceutical manufacturing as well as use in firefighting foams. As a result of growing use and their stable nature, PFAS are now ubiquitous in the environment showing bioaccumulation and adverse health effects in wildlife and humans. Although PFAS are currently being studied worldwide, little information on PFAS concentration in Ireland's marine environment exist. The main objective of this study was to determine PFAS concentration in transitional and marine waters along Dublin Bay and pinpoint areas where the existing concentration of these compounds, or slight increases could pose an environmental problem. Marine samples were treated with sodium thiosulfate to reduce free chlorine prior to analysis. Water samples were extracted by solid phase extraction (SPE) using Oasis WAX (500 mg). The extract was analysed by High performance liquid chromatography (HPLC) using an Agilent Zorbax Eclipse Plue C18 column (3.0 x 50 mm; 18µm) and an identical guard column. For detection, HPLC was coupled with a triple quadrupole mass spectrometer. Results confirm the presence of both legacy and novel PFAS in transitional waters entering Dublin Bay, with fluctuations in concentrations observed in upper and lower estuary sites along the river Liffey.

The highest concentrations detected were 1393.7 and 1217.9 ng/l at upper estuary sites for perfluorooctanoate (PFOA) and perfluoroundecanoate (PFUdA) respectively. Levels of PFOA, a legacy PFAS, decreased from upper to lower stream sites with a concentration of 57.7 ng/l at the final sampling site in Dublin Bay. The novel PFAS perfluoropropoxypropanoic acid (GenX) was detected at upper estuary sites with the highest concentration of 37.8 ng/l, this decreased downstream at lower estuary sites to <25ng/l.

The two final sampling sites were located upstream and downstream of Ringsend wastewater treatment plant (WWTP). Five of the seven analytes detected at these two sites were found to increase downstream of the WWTP, these analytes include GenX which increased from 18.8 to 23.9 ng/l, and perfluorohexanoic acid (PFHxA) which increased from 95.8 to 157.9 ng/l downstream of the WWTP. PFUdA concentration was found to decrease from 1066.3 to 582.7 ng/l between these two sites

### **3.05.P-Th230 An Investigation into Apolar Extractable Organofluorine**

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Current fluorine mass balance (FMB) studies employ extraction procedures designed to extract polar and semi-polar per- and polyfluoroalkyl substances (PFAS), such as perfluoroalkyl acids. However, organofluorine substances encompass a wide range of structures with equally diverse properties. Non-polar PFAS, such as bromotetrafluoro biphenyl, fluorinated liquid crystal monomers and fluorinated polycyclic aromatic hydrocarbons, are known to exist, but are rarely considered in FMB experiments. Moreover, the recent discovery of unidentified extractable organofluorine (EOF) in the blubber of a Greenland killer whale suggests that unknown lipophilic fluorinated substances are already accumulating in wildlife. Therefore, to obtain a complete picture of the organofluorine burden in the environment, alternative extraction procedures must be considered.

The current work aimed to investigate the prevalence of apolar EOF in a diverse range of environmental samples. We started by performing spike/recovery experiments in fish muscle and/or diatomaceous earth using PFAS with a diverse range of physical-chemical properties, namely: 1) perfluorooctane sulfonic acid (PFOS), 2) 1H,1H,2H,2H-perfluorodecyltriethoxysilane (a fluorinated silane used in cosmetics), 3) Scotchgard™ (a side chain fluorinated polymer, SFP), and 4) a textile treated with a C<sub>8</sub>F<sub>17</sub>-SFP. Sample extraction was carried out with polar solvents typically used for EOF determination, followed in series by a re-extraction with hexane to measure apolar EOF. Total fluorine (TF) was measured for neat samples and for extracted (“spent”) samples. Combustion ion chromatography was employed for all analyses. Following the spike/recovery experiments, the procedure was repeated on liver and blubber from marine mammals, as well as dust and municipal wastewater treatment plant sludge.

Overall, spike/recovery experiments demonstrated that SFPs are only partially extractable using both polar and non-polar solvents, which might be the case for several PFAS subclasses. In unfortified samples, significant quantities of both polar and apolar EOF were observed. Moreover, notable levels of fluorine were still present in samples after the two consecutive extractions, attributable to either inorganic fluorine and/or unextractable organofluorine. This study highlights the need to characterize unknown apolar EOF and develop novel extraction and analytical methods for the identification of neutral organofluorines.

### **3.05.P-Th231 Expanding Per- and Polyfluoroalkyl Substances Coverage in Nontargeted Analysis Using Data-Independent Analysis and IonDecon**

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The FluoroMatch suite of open-source software tools simplifies suspect and nontargeted screening of PFAS compounds. It automates file conversion, chromatographic peak picking, blank feature filtering, PFAS annotation based on precursor and fragment masses, and annotation ranking. The software library currently contains 15,643 PFAS fragmentation patterns based on rules derived from standards and literature, and the software automates a process to add more compounds to the PFAS library. FluoroMatch Flow directly processes vendor files and includes a systematic scoring framework to communicate confidence for every feature, alongside reporting confidence levels via the Schymanski schema.

IonDecon is the latest FluoroMatch software tool, it deconvolutes All Ions Data Independent Analysis (DIA) data files to correlate fragment ions with precursor ions. It takes DIA data to generate an open source Data Dependent Acquisition (DDA) formatted file, which can be used in any downstream nontargeted analysis workflow. The deconvoluted All Ions spectra retained the most abundant peaks also observed in DDA, while filtering out much of the artifact peaks. In a neat solution, annotation of PFAS standards using IonDecon and All Ions DIA had the same false positive rate as DDA. However, IonDecon can retain some false fragments in complex samples, moderately increasing the false discovery rate. Overall, incorporating DIA All Ions fragmentation (AIF) and IonDecon can enhance the MS/MS coverage of PFAS (more than tripling the number of annotations in domestic sewage) without resorting to a time-consuming DDA workflow.

### **3.05.P-Th233 Investigating The Extent of Legacy Per- and Polyfluoroalkyl Substances Contamination in English Surface Waters: An Environment Agency Water Quality Archive Investigation**

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Poly- and Perfluoroalkyl Substances (PFAS) are a large group of persistent and bioaccumulative chemicals found in many consumer products. Their unique physicochemical properties of thermal and chemical inertness and water and grease-repellency grant them applicability in many products including non-stick cookware, food packaging, fire-fighting foams, electrical products and others. Subsequently, PFAS enter wastewater and runoff streams and eventually end up in rivers and estuaries. Perfluorooctanoic (PFOA) and Perfluorooctanesulfonic acid (PFOS) also known as legacy PFAS have been widely monitored since the early 2000s as they are present in humans and wildlife, and are associated with an array of toxic effects. They are also listed in the UNEP Stockholm Convention as POPs: PFOA under Annex A, ‘elimination’, and PFOS under Annex B ‘restriction’. However, little is known about their use, lifecycle, sources and occurrence in UK surface waters. Using surface water monitoring data focusing on PFOS and PFOA from the Environment Agency’s Water Quality Archive, collected

over the period 2019-2022, the aim was to investigate the spatial distribution of these chemicals in the English aquatic environment and identify patterns of their occurrence related to land use.

Given the large number of monitoring sites, the Water Quality Archive provides a foundation for detailed insights into the spatial differences in chemical concentrations. Moreover, clustering analysis for mixed data (k-prototypes) in conjunction with a GIS approach was applied to link land use (Rural and Urban Areas, Non-irrigated Arable Land and Coasts and Estuaries), PFAS occurrence and suspected PFAS-rich nearby sources.

The clustering output successfully separated sites with relatively higher signals from sites with lower concentrations. Most of the higher signal samples were taken from urban areas which were isolated in one cluster. This confirmed that densely populated areas drive higher PFAS emissions. Sites in the vicinity of suspected PFAS point sources of pollution were isolated (airports, firefighting stations, wastewater treatment plants). Overall, this study is useful for chemical risk assessments to effectively predict PFAS hotspots and develop appropriate ways to mitigate PFAS pollution in heavily contaminated areas. Lastly, the results of this study provide the basis for more specific catchment-based monitoring to understand PFAS occurrence and sources in English surface waters.

### **3.05.P-Th234 Monitoring Produced Gases From PFAS Removal Technologies Using Thermal Desorption Coupled To Gas Chromatography/Mass Spectrometry**

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Many per and polyfluoroalkyl substance (PFAS) removal technologies seek to breakdown the compounds into small components which can be captured or neutralized. Incineration of PFAS, and PFAS containing products like aqueous film forming foams (AFFF), is a common disposal method and aims to degrade PFAS species into HF, CO<sub>2</sub> and water. However if this process does not happen under the correct conditions products of incomplete destruction (PIDs) are created. This is also true of other novel techniques which are now being researched and used to try and managed PFAS. Often PIDs are smaller fluorinated species and have their own detrimental effect upon the environment such as acting as greenhouse gases or ozone depleting substances. This means that PFAS destruction must be monitored in a meaningful way.

Thermal desorption coupled to gas chromatography and mass spectrometry (TD-GC-MS) is commonly applied to monitoring of hazardous organic compounds in ambient air. The technique is well suited to monitoring ultra-volatile species and enables detection of compounds at low ppt levels from canister or online samples.

In this study we will share data generated using a Markes UNITY-CIA Advantage-xr preconcentrator system and the 8890/5977 for GC/MS analysis. The matrix from incineration based sources can be challenging due to high levels of CO<sub>2</sub> and water which is a challenge for sample injection, separation and compound detection. The data will show that canister sampling offers a robust approach to performing monitoring of ultra-volatile perfluorocarbon species which are expected to be produced at destruction sites and enable detection limits as low as 1 pptv.

### **3.05.P-Th235 Quantitation of different compound classes in drinking water to comply with EU regulations by LC-MS/MS workflow and direct injection**

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Drinking water analysis is important and all water supply companies need to ensure that the final water product they send out into supply networks is safe and complies with state and country regulations, for example, EU directive 2020/2184. This directive establishes detection limits for a whole series of compound classes that are considering harmful for human healthy and restricted. This work demonstrates the performance of both the SCIEX QTRAP 6500+ and SCIEX QTRAP 7500 systems, to be able to answer the challenge. High sensitivity and robustness of the methods achieve levels for all legislated compounds without need of extensive sample preparation and sample preconcentration step, simply by direct injection.

Standard SOPs will be provided with the necessary information for the analysis of following compound classes:

- Haloacetic acids (HAA) where the sum of 6 selected HAAs must not exceed a concentration of 60mg/L.
- Pharmaceuticals and personal care compounds (PPCPs), including azithromycin and diclofenac where each compound must not exceed a concentration of 100ng/L.
- Polyfluoroalkylated compounds (PFAS) where each PFA must not exceed a concentration of 100ng/L.
- Polar pesticides where each compound (Glyphosate, Gluphosinate and AMPA) where each PFA must not exceed a concentration of 100ng/L.
- Acrylamide where must not exceed a concentration of 100ng/L
- b-Estradiol where must not exceed a concentration of 1ng/L
- 4-nonylphenol where must not exceed a concentration of 300ng/L
- Bisphenol A where must not exceed a concentration of 2.5mg/L

All methods demonstrated high sensitivity and robustness. Most important, possible combination of different compound classes to one HPLC method and easy of change between different methods.

### **3.05.P-Th236 Utilizing Ion Mobility to Enhance Targeted and Non-Targeted Analysis of Per- and polyfluoroalkyl substances (PFAS) from a Landfill Leachate Sample**

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Per- and polyfluoroalkyl substances (PFAS) are a group of industrial compounds that have been identified as a class of environmental contaminants of high concern due to their negative health effects and widespread presence in the environment. As new regulations on the use and manufacture of legacy PFAS are imposed, alternative replacement PFAS compounds have emerged. Current targeted methods focus on a short list of legacy and emerging PFAS and are therefore not comprehensive enough for measuring the potential PFAS contamination in environmental samples. Non-targeted analyses (NTA) with high-resolution mass spectrometry (HRMS) is a promising technique for the discovery and identification of new PFAS. Here, HRMS was coupled with liquid chromatography and ion mobility separation on the SELECT SERIES™ Cyclic™ IMS QTOF mass spectrometer (Waters Corporation) to improve the number of detected features in a complex environmental sample: landfill leachate. As PFAS may be present at low levels in complex samples, a data independent acquisition was utilized to get as much information as possible in a single injection. Detected peaks were first compared to an internal HRMS PFAS library for identification. Identifications were filtered based on metrics like accurate mass, retention time error, diagnostic fragments and measured collision cross section (CCS). In the sample, several legacy PFAS including perfluoroalkyl sulfonates, perfluoroalkyl carboxylic acids, and fluorotelomer sulfonates were identified. For the discovery of PFAS not in the suspect library, IMS adds an additional advantage as this class of compounds have lower CCS values compared to other compound classes of similar *m/z*. In this study, filtering peaks based on their observed drift time and *m/z* revealed polyhalogenated species that were in the leachate sample even at low intensity levels. A series of PFAS previously unreported were tentatively identified in the leachate samples. As authentic standards for new PFAS are difficult to find, the certainty of proposed structures benefit greatly from the data generated with LC-IMS-HRMS.

### **3.05.P-Th237 Extending PFAS coverage and sensitivity in a direct injection UPLC-MS/MS method for water matrices based on EU and UK Drinking Water regulations**

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Per- and Polyfluoroalkyl Substances (PFAS), are extremely persistent in the environment. Their production and use have resulted in severe contamination of soil, water and food. To protect public health, advisory and regulatory limits continue to be created and updated. Consequently, routine PFAS analysis has become challenging as not only low detection limits are required, but extensive compound coverage is a prerequisite nowadays.

The purpose of this work was to demonstrate a direct injection method to fulfil the requirements of a list of 47 per-and polyfluoroalkyl substances (PFASs) by the UK Drinking Water Inspectorate (DWI) and the 20 PFAS contained in the 2020 EU Drinking Water Directive (DWD) totaling 48 analytes. The work was carried out using an ACQUITY™ Premier UPLC system coupled to the XEVO™ TQ Absolute tandem quadrupole mass spectrometer using a UniSpray™ ion source. Sub-ng/L Method Detection Limits (MDLs) were achieved for all 48 analytes using a 50 µL injection volume. Method robustness was shown with validation batches analyzed over three days in drinking water, surface water and river water.

Improvements in both the analytical and isolator column technologies demonstrated in this work, as well as enhancements in negative ion sensitivity from the Xevo TQ Absolute Mass Spectrometer are helping to support ongoing efforts in PFAS analysis. This allows for easier, more robust, and accurate options as PFAS analysis continues into the future.

### **3.05.P-Th238 Screening of Legacy Precursor Per- and Polyfluoroalkyl Substances (PFAS) and Potential Precursors in Curtains, Sofas and Carpets Fabric Samples**

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Per- and polyfluoroalkyl substances (PFAS) are a huge group of manmade compounds used for example as water and oil repellents to protect textiles and fabrics. Due to a growing concern around these compounds, industries introduced new PFAS varieties. Various PFAS are therefore found in fabric samples, for example, due to contamination with dust, other manufactured products, storage or transport. Possible restrictions concern only legacy PFAS, and not suspected or recently identified compounds, meaning that the environment and the population are still potentially exposed to unknown chemicals. This study focusses on the synthesis of newly synthesized PFAS precursors and screen them together with conventional PFAS in 32 curtains, sofas and carpets fabric samples. Synthesized compounds include *N*-sulfo propyl perfluorohexane sulfonamide, short-chain perfluoroalkane sulfonamide derivatives (C4 and C6), namely *N*-alkyl perfluoroalkane sulfonamide and *N*-alkyl perfluoroalkane sulfonamidoacetic acid, *N*-trimethylammonio propyl perfluoroalkane sulfonamide (C4, C6 and C8), 6:2 fluorotelomer sulfonamide, 6:2 fluorotelomerthia propanoic acid, *N*-dimethyl ammonio propyl perfluoroalkane sulfonamide (C4, C6 and C8) and *N*-sulfo propyl dimethylammonio propyl perfluorohexane sulfonamide propyl sulfonate. Samples were extracted two times with methanol before clean-up using Envi-carb. Samples were analysed by LC-MS/MS. Legacy PFAS were observed in low concentrations for most of the samples but in some of them, particularly in fabrics based on recycled materials, concentrations were much higher. For instance, PFOS was identified in two samples with a concentration of 56 ng/g (22335 ng/m<sup>2</sup>) and 0.87 ng/g (1660 ng/m<sup>2</sup>), considerably higher than EU limit of 1 µg/m<sup>2</sup>. Various



newly synthesized precursors were also identified, especially in the carpet samples. For instance, 2:2 FTS (4.3 ng/g – 8223 ng/m<sup>2</sup>) and MeFBSAA (0.18 ng/g – 783 ng/m<sup>2</sup>) were detected. These preliminary results suggest that PFAS not usually screened can also be present in carpets. These samples will also be analysed for the volatile PFAS by gas chromatography.

### **3.05.P-Th239 Per- And Polyfluorinated Alkyl Substances (PFAS) in Car Interior Materials**

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Per- and polyfluorinated alkyl substances (PFAS) is a chemical family with several thousands different compounds that have been used in numerous different applications due to their water- and grease repellent properties. The usage of PFAS includes a vast number of consumer product applications, such as in cosmetics, textiles, leather, paperboard, waxes, and electronic. Increasing knowledge and awareness about the toxic and bioaccumulative properties of PFAS has led to increasingly stricter regulations and voluntary phase-out not only of single PFAS compounds but also of the PFAS family as a whole. Though a number of recent studies address PFAS in consumer products, there is still only a few studies about PFAS in car interior materials. PFAS in car interior materials implicates an environmental risk at end-of-life when destined to landfills, and also pose a risk for human health considering the large number of hours many drivers spend in cars. Sources for PFAS in car interior materials may be intentionally added PFAS for grease- and water repellent performance, PFAS present in the original feedstock of materials, or stem from recycled materials used in the production. Investigations of PFAS contamination patterns in car interior material can provide increased understanding of contribution from both intentionally added PFAS as well as the role of recirculation and background contamination.

In this study, samples from car interior materials including foam, artificial leather, car seat textile, and plastic components from 2022-2023 were extracted and analyzed for a broad range of PFAS compounds (persistent PFAS, intermediates, and precursors). Extraction was performed using ultrasonication with hexane:isopropanol and acetonitrile:methanol. Analysis was performed using liquid chromatography tandem mass spectrometry (LC-MS/MS).

### **3.05.P-Th240 Fate of PFAS in dune infiltration systems**

*Elvio D Amato, KWR Water Research Institute, Netherlands*

Per- and polyfluoroalkyl substances (PFAS) are a very broad and diverse group of chemicals that pose a risk to human and environmental health. Several processes contribute to the spreading of PFAS in the environment, including atmospheric transport via sea-spray aerosols (SSA), which are known to contain elevated levels of PFAS. Due to their amphiphilic properties, PFAS are impervious to conventional treatment processes used for drinking water production, including dune infiltration. Furthermore, deposition of SSA may potentially introduce additional PFAS in coastal dunes, and pose a risk to drinking water sources. In this study, we investigated the occurrence of PFAS in SSA, soil, and groundwater in a dune infiltration area. The presence of additional PFAS that are not included in targeted methods was also investigated using complementary suspect and non-targeted screening (SNTS) analysis performed on SSA samples. Strong relationships were found between PFAS and Na measured in air samples, indicating that SSA was likely a major source of atmospheric PFAS. The general trend indicated increasing concentrations of carboxylic acids from C5 to C8 followed by decreasing concentrations from C8 to C11. Sulfonic acids included PFOS, PFHxS, PFBS and PFHpS. PFOS and PFOA were by a significant margin the most abundant compounds in SSA. Precursors such as 6:2 FTS and FOSA were also detected in SSA, however, no correlations with Na were observed. SNTS analysis performed on SSA samples identified several compounds (confidence level 2-3 according to Charbonnet et al. (2022)) that were not included in the targeted method, such as MeFBSAA and fluorotelomers, which may contribute to undetected contamination in the dunes, and formation of more stable PFAS upon degradation of precursors. Increasing PFAS levels were measured in groundwater upon infiltration in coastal dunes. Contamination in soil and groundwater appeared to be consistent with potential contributions from SSA deposition, however, further research is needed to quantify the input of PFAS from SSA deposition, and understand transport mechanisms of PFAS in the saturated and unsaturated zones. SNTS analyses tentatively annotated several unmonitored PFAS in SSA, including precursors. Complementary analyses aiming at quantifying the total PFAS burden should be performed to investigate the potential role of PFAS precursors in coastal dunes.

### **3.05.P-Th241 To Isolation and Beyond: A New Mixed Mode Column Approach for PFAS Chromatography**

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Per- and polyfluorinated alkyl substances (PFAS) are one of the most commonly discussed and researched environmental contaminants. This is due in part to how widespread and impactful PFAS contamination has become, but also from increasing media awareness. Initially, most focus was on monitoring PFAS in water, mainly drinking water, but the prominence of these compounds in everyday items have expanded analysis to other matrices such as food, cosmetics, and human biofluids. As more research is focused on PFAS exposure and impacts, analytical techniques have been advancing as well to help progress research and monitoring efforts.

One of the constantly changing areas in PFAS analysis is the list of PFAS compounds that are being targeted for. One trend impacting this is the movement of manufacturers from using long chain PFAS to short chain and ultra-short chain PFAS. As the chain length shortens, chromatography can become more complicated, not only from a perspective of the analytical column the sample is separated on, but also regarding the functionality of the isolator column that is crucial to delaying PFAS interference from the LC system. These compounds require advancements in chromatographic techniques to properly detect and quantify in samples.

Faced with this challenge, a new mixed mode column (XBridge™ Premier BEH™ C18 AX Column) was evaluated as both an isolator column and an analytical column to provide better retention of short chain and ultra-short chain PFAS. This presentation will demonstrate how this column was applied in both cases to not only delay chromatographic interference, providing accurate sample quantitation, but also to extend the possible list of PFAS analyzed in a single injection to include PFAS chain lengths.

### **3.05.P-Th242 Uncovering the PFAS Complexity: A powerful IMS-QTOF Workflow for Biota Analysis combining Targeted and Non-target Approaches**

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With their widespread presence in the environment and organisms, and their persistent, bioaccumulative, and toxic (PBT) properties, Per- and Polyfluoroalkyl Substances (PFAS) pose a significant challenge as organic micropollutants. The vast array of commercially available PFAS compounds and their transformation products necessitate tight monitoring within environmental compartments. This study aims to the development of a combined targeted and non-target workflow for the thorough characterization of PFAS in complex environmental matrices, particularly biota, by incorporating trapped ion mobility spectrometry (TIMS) into LC-HRMS.

Biota extracts were prepared by a generic protocol for the simultaneous extraction of 56 PFAS compounds from various sub-groups. RPLC was coupled to a novel heated electrospray source (HESI) for analyzing the extracts in negative ion mode on a timsTOF Pro 2 (Bruker). The targeted workflow utilized bbCID (broadband collision-induced dissociation) in data-independent acquisition (DIA), while the non-target workflow employed PASEF (parallel accumulation and serial fragmentation) in efficient data-dependent acquisition (DDA). The CCS-aware target analysis incorporated information for a list of 60 PFAS compounds, including elemental composition, retention time, MS1 and MS2 qualifier ions, and ion mobility-derived collision cross section (CCS) values.

The results of the targeted screening demonstrated improved sensitivity and lower PFAS detection limits due to HESI, along with higher quality for the full-scan MS and bbCID MS/MS spectra supported by the ion mobility filtering. Adding CCS values enhanced the identification confidence. In the non-target workflow, raw data was transformed into a comprehensive feature table, and the detected features were filtered by Kendrick mass analysis. A PFAS suspect list with 5,000 compounds was employed for further annotation. *In-silico* prediction of MS/MS spectra and CCS prediction aided the identification of suspected compounds. Notable examples highlight the identification of suspected features as PFAS-related compounds, supported by the extensive MS2 coverage provided by PASEF.

This combined approach contributes to a better understanding of the chemical universe of PFAS in the environment and plays a crucial role in safeguarding the environment, wildlife, and human health in a One Health approach.

### **3.05.P-Th243 Fate of Per- and Polyfluoroalkyl Substances in a Waste-to-Energy (WtE) Plant – Transport and Distribution in WtE Residues**

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Per- and polyfluoroalkyl substances (PFAS) are a ubiquitous group of man-made chemicals used in various consumer products. Given their widespread use, some products containing PFAS will eventually end up in waste streams which in many European countries are processed in Waste-to-Energy (WtE) facilities. The fate of PFAS in full-scale incineration is largely uncertain, and there is a divergence between lab-scale and full-scale studies, highlighting the need for a comprehensive investigation of the fate of PFAS in WtE.

The present work is the first study assessing the fate of PFAS in a WtE plant and investigating the distribution of PFAS in solid, gaseous, and liquid residual fractions. Bottom ash, filter ash, boiler ash, air pollution control residue, gypsum, flue gas (before wet flue gas treatment and in the stack), condensate and treated process water from the in-house water treatment plant was sampled at an operating WtE plant. The plant was incinerating a mixture of household waste (60%) and industrial waste (40%). All samples were analyzed for 18 individual PFAS by LC-qTOF/MS.

PFAS was detected in filter ash and bottom ash, but not in boiler ash, air pollution control residue or gypsum. PFAS was observed in both condensate and treated process water. Interestingly, the total concentration of PFAS in treated process water was 25-100% higher than that in condensate, indicating that there are additional sources of PFAS from the wet flue gas treatment to the treated process water.

PFAS was detected in both untreated flue gas and in the stack. A reduction of the total concentration of PFAS was observed when comparing concentrations before and after the wet flue gas treatment, on average -35% ±10%. This suggests that the wet flue gas treatment can transfer some PFAS from the flue gas into other residual streams.

The total annual release of PFAS via WtE residues from this plant was estimated to be between 7 and 20 g, equally distributed between bottom ash, treated process water and stack flue gas. In comparison, the annual release via leachate from the temporary waste stockpile at the same plant was estimated to be 33 g per year.

This study provides the first insights into the transport and distribution of PFASs in a WtE plant. Moreover, these results demonstrate that some PFAS are not fully degraded by the high temperatures during WtE processes and can be emitted from the plant via bottom ash, treated process water, and flue gas.

### **3.05.P-Th244 Electrochemical Degradation of Perfluorinated Organic Compounds (PFAS) in pH-Controlled media using Boron-Doped Diamond (BDD) Flow Cell**

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Water contamination by Per- and polyfluoroalkyl substances (PFAS) presents a pressing environmental concern, given their persistent and bioaccumulative nature, posing threats to ecosystems and human health. PFAS, integral to numerous industries, consequently, PFAS experiences substantial release into water sources. Conventional treatment methods challenge incomplete remediation, by-product accumulation and high energy and chemical consumption. The utilization of the BDD-flow-cell promises a safe, efficient, and cost-effective method for PFAS removal emphasizing the importance of minimizing auxiliary substances and by-products to prevent secondary water pollution. This research investigates a promising electrochemical degradation (ED) technique employing a Boron-Doped Diamond (BDD) flow-cell under varying pH conditions. BDD is utilized to overcome these challenges due to its high chemical and electrochemical stability, especially the electrochemical resistance even under severe conditions.

Our aim is to investigate the PFAS-ED method, targeting various PFAS chain lengths and moieties. Employing LC-MS/MS analysis, we elucidate the electrochemical degradation (ED) process, providing comprehensive mass balances for degradation products. Model water experiments, comprising Perfluorooctanoic acid (PFOA), Perfluorooctanesulfonic acid (PFOS), and Perfluorobutanesulfonic acid (PFBS), will be conducted using a BDD electrode flow-cell system. The flow-cell, with a BDD working electrode and a stainless-steel counter electrode, circulates electrolyte-PFAS solution, optimizing the current density for maximum PFAS degradation. Exploring different pH media, including acidic, basic, and neutral electrolyte-PFAS solutions, will help to determine the optimum conditions for ED efficiency.

Preliminary results reveal higher degradation efficiency in basic media, followed by neutral, while acidic media exhibits the least ED efficiency. Our research investigates the role of pH in the ED process, elucidating the impact of OH<sup>-</sup> and H<sup>+</sup> ions for optimal selectivity and efficiency. This study not only advances ED methods for PFAS remediation but also provides insights into reaction mechanisms for sustainable water purification. Future work will implement the optimized ED method for real water treatment.

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### **3.05.P-Th245 Solving the PFAS Challenge: Comprehensive Screening of Environmental Samples against 1000s of Compounds in a Single Run**

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PFAS are known as “forever chemicals” due to their persistent, bio-accumulative, toxic (PBT) properties and ubiquitous presence in the environment and organisms. Ca. 5000 PFAS are marketed worldwide, making their systematic environmental monitoring an extremely challenging task. On top, there is a lack of reference standards or spectral libraries, and there are plenty of isomers. Adding trapped ion mobility spectrometry (TIMS) to UHPLC-HRMS allows for comprehensive monitoring of organic micropollutants in environmental matrices such as water and soil.

Water samples were spiked with sets of PFAS compounds or taken from common household and environmental water resources. Data independent acquisition was performed on a high-resolution mass spectrometer equipped with ion mobility. Kendrick mass defect (KMD) analysis filtered potential PFAS from the matrix background, based on the fluorine content (repeating CF<sub>2</sub> units). Spectra were compared with either a spectral library of available PFAS standards in a targeted workflow or with the Norman network and NIST suspect lists of 5000 entries for non-targeted analysis. The Norman and NIST suspect lists contain information about the elemental composition and the InChI structures for PFAS which were compared with the exact mass, MS/MS fragmentation and CCS values of the experimental data for an automated and untargeted identification of all PFAS present in the sample.

The ion mobility feature of the system was utilized for several purposes. First, it can separate coeluting isobars and isomers. Second, the TIMS filter results in higher sensitivity and lower detection limits of the targeted PFAS as well as significantly higher quality of full-scan MS and b/cCID MS/MS spectra. Finally, collisional cross sections (CCS) as additional identification criteria enhanced the identification confidence with is based on the exact mass, diagnostic fragmentation ions and the isotope

pattern fit. The wide-scope suspect screening of real-life samples against the suspect lists proved to be a comprehensive approach for a fast and efficient identification and quantification of PFAS against the total set of 5000 compounds in complex environmental matrices. Therefore, it will assist in understanding the chemical universe of PFAS in the environment and protecting environment, wildlife, and human health.

### **3.05.P-Th246 Ion Mobility Filtering for Non-Targeted Analysis of PFAS from Environmental Samples Collected at a Ski Resort**

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Perfluoroalkyl substances (PFAS) are a group of commonly used compounds, known particularly for their hydrophobic, non-stick properties. Their unique chemistry led to their use in ski waxes. While competition rules have recently banned the use of PFAS-containing ski wax, the persistence of PFAS means they could still be detected for years. Given the hazards and concern about PFAS contamination, we investigated if PFAS could be detected at a ski area that supports a high-level race program. While previous studies have looked at targeted analysis for known PFAS, this study used high-resolution mass spectrometry (HRMS) and ion mobility to look for new and unexpected PFAS. Samples were collected from a variety of locations within a ski area in New Hampshire to investigate the trends of PFAS in this type of environment. Data collection was done with ion mobility enabled data-independent acquisition on a SELECT SERIES™ Cyclic™ IMS (Waters Corporation). The added dimension of ion mobility can be used for spectral clean-up, which aids in the elucidation of unknown compounds as well as confirmation of known target compounds. Detected peaks were first compared to an internal HRMS PFAS library for identification. In the extracts, several legacy PFAS including perfluoroalkyl sulfonates, perfluoroalkyl carboxylic acids, and fluorotelomer sulfonates were detected. Unknown peaks were selected for further scrutiny based on their detected drift time in the ion mobility dimension. Previous studies have shown that the CCS values of per- and poly-fluorinated compounds are lower than compounds of similar *m/z*. An ion mobility filter was created based on this knowledge and applied to a list of detected peaks to select for possible PFAS. Using this filter, a series (C9-C24) of polyfluorinated carboxylic acid compounds with one hydrogen substitution in the carbon chain was tentatively identified. The distribution for this series of compounds was not consistent, with longer chains found in samples from the base of the ski slopes and the shorter chains in the snowmaking retention pond. While authentic standards were not available for many of the tentative identifications, two standards were purchased and compared with experimental data to confirm proposed structures.

### **3.05.P-Th247 Pushing PFAS Possibilities: The hunt for Ultra Sensitivity to Reach ppq EPA Health Advisory Levels**

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PFAS are well known chemicals with a variety of commercial and consumer uses, characterized by their extreme stability and structural diversity. PFAS are highly persistent and toxic contaminants that accumulate in humans, animals, and the environment. Detection requirements for PFAS have been getting more challenging as advisory and regulatory limits continue to be created and updated. In June 2022, the US EPA tightened its health advisory levels (HALs) in drinking water from 70 ng/L to 0.004 ng/L for PFOA and 0.02 ng/L for PFOS. Additionally, final HALs were set for PFBS at 2000 ng/L and HFPO-DA (commonly referred to as GenX) at 10 ng/L. The near zero PFOA and PFOS levels pose analytical challenges regarding instrument sensitivity and sample preparation conditions to limit contamination. This goal of this work was to show with proper care during sample preparation and analysis using a highly sensitive mass spectrometer, these near zero limits are possible to detect.

All samples were prepared in a typical shared laboratory environment, taking extreme care to screen all consumables and reagents for PFAS contamination before use. 250 mL water samples were extracted using Oasis™ WAX SPE Cartridges, followed by analysis using a highly sensitive mass spectrometer.

This presentation will discuss best practices for sample preparation to reduce contamination as well as the minimum level of contamination achieved during these experiments. Additionally, information on the Limit of Quantitation (LOQ) for each compound and method recovery values will be shared.

### **3.05.P-Th248 Performance of Different Sorbents on the Removal of Per- and Polyfluoroalkyl Substances from Aqueous Firefighting Foam Impacted Ground- and Wastewater**

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PFAS containing firefighting foams are typically applied on sites with increased risk of flammable liquid (Class B) fires, such as air bases or refineries. In fire fighting training activities as well as fire emergencies PFAS-containing Aqueous Film Forming Foams (AFFF) have been and are used. The PFAS fluorosurfactants in these foams create a thermally stable film that covers the flammable liquid. Although AFFF mitigates Class B fires, the remnants of these fires and training activities poses an environmental risk as large volumes of PFAS containing wastewater is produced, which can possibly contaminate soil, groundwater and surface water. The aim of this research is to assess the typical PFAS constituents of AFFF impacted waste and groundwater and to study the intrinsic removal of these molecules from these field derived water matrices by various sorbents in a batch setup.

### **3.05.P-Th249 Developing Targeted and Untargeted Liquid Chromatography-Tandem Mass Spectrometry Methods for Analyzing PFAS in Potable and Wastewater Samples**

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Per- and polyfluoroalkyl substances (PFAS) are a group of man-made compounds with a wide range of applications from industry to everyday life (e.g., coatings to enhance corrosion resistance, emulsion polymerization, cookware, food packaging, etc.). Although the use of some PFAS has been restricted, their ubiquity has resulted in significant accumulation within the environment. Many drinking (potable) water and wastewater plants have been employing methods intending to reduce PFAS concentrations in environmental media. However, little research has been devoted to studying PFAS degradation products and their potentially adverse impact on the environment.

Our work focuses on developing the sample preparation, as well as both targeted and non-targeted liquid chromatography-tandem mass spectrometry method. The targeted method is improved from the 4<sup>th</sup> Draft EPA method and employed on Agilent 1100 liquid chromatography coupled to a SCIEX 6500 triple quadrupole mass spectrometer, while the non-targeted method is developed on a Waters Acquity liquid chromatography coupled to a Waters Synapt G2-Si quadrupole time of flight mass spectrometer equipped with a ion mobility spectrometry.

Preliminary results of the targeted method include the assessment of the instrumental minimum detection limits, extraction recoveries, and instrumental carryover. The instrumental minimum detection limits for a set of 40 PFAS compounds ranged between 0.05 and 2.0 ng L<sup>-1</sup>. At the same time, the recoveries from aqueous and solid samples met the requirements of the 4<sup>th</sup> Draft EPA method for detected PFAS. Preliminary results of the non-targeted method yield less than 5 ppm mass accuracy of the standards and isotopically-labeled standards.

This study is relevant to the worldwide community because it focuses on developing a method for analyzing PFAS present in aqueous and (bio)solid samples. The non-targeted method will provide higher degrees of confidence when assigning annotations to unknown features from environmental samples by combining retention time, mass accuracy, and collision cross sections from the ion mobility spectrometry. This project is also directed towards developing machine learning algorithms that will aid the identification of unknown features in non-targeted spectra.

### **3.05.P-Th251 An ultra-high sensitivity and robust analysis of PFAS compounds in multiple water sources.**

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PFAS compounds are becoming increasingly ubiquitous in our environment due to overuse and their lack of breakdown, ensuring that this will be a challenge for decades to come. Therefore, it is imperative to provide rigorous and sensitive analytical testing to regulate these compounds and try to limit their possible effects on human health

As the limits for PFAS compounds continue to be reassessed and reduced it becomes increasingly important to provide methods which achieve limits of detection which are as low as is reasonably possible

It has been well documented that when performing PFAS analysis contamination and interference can be large challenges of a successful analysis due to the numerous sources of these compounds and the interferences which can be observed. Here, we detail the steps which we recommend being taken to ensure the analysis of PFAS compounds is as simple and challenge free as possible:

Overall, it is possible to achieve ultra-high levels of sensitivity for PFAS compounds down to 0.2 ng/L when using the SCIEX 7500 System. Excellent levels of precision, accuracy and linearity have been achieved for all compounds analyzed. Detection limits down to 0.06 ng/L in three different water matrices. The importance of reducing interferences and contamination is paramount to a successful PFAS analysis. And long term stability of the method will be demonstrated in this poster.

### **3.06 Application of Biomonitoring Approaches to Support Surveillance of Chemical Exposure in the Environment**

#### **3.06.T-01 On-site Application of Biological Early Warning Systems in combination with high resolution Online-Chemical Monitoring**

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Detection of problematic substances in wastewater effluents by using traditional toxicity testing approaches including ecotoxicological bioassays or chemical analysis with discrete effluent samples, poses a difficult and costly task for environmental researchers. A continuous monitoring approach using biological early warning systems (BEWS) coupled with online chemical analysis to indicate micropollutant dynamics in the effluent could be an interesting combination to address this challenge. In this study, three selected BEWS with *Chlorella vulgaris*, *Daphnia magna* and *Gammarus pulex* as test organisms, were operated in a full-scale municipal wastewater treatment plant (WWTP) in parallel with an automated online HPLC high resolution mass spectrometric system (MS2Field) to monitor wastewater effluent. During the five weeks of operation, the

BEWS showed alarms and deviations from normal behavior, while target and non-target screening indicated micropollutants at elevated concentrations in the treated wastewater. In the second week, increased *Gammarus* activity was registered during a rainfall event, which could be related to the change in abiotic parameters. In the third week, the presence of several suspect compounds was correlated with behavioral responses that led to alarms. In addition to the suspect compounds, a pesticide not authorized in Switzerland was identified by non-target analysis. This pesticide occurred in concentrations close to literature EC50 concentrations. In subsequent verification tests, test organisms in the BEWS were exposed to this pesticide in the same concentration range as measured in the effluent. This provided evidence that the measured concentration of the pesticide in the WWTP effluent most likely induced the organisms' response. Overall, the integration of biological and chemical monitoring with continuous and high temporal resolution provided a promising approach to identify critical contamination hotspots in WWTP effluent with relatively low cost and maintenance.

### 3.06.T-02 Development of a Holistic Approach for River Health Assessment: from Bioindicators to the Ecosystem

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Freshwater ecosystems are essential for the planet. Despite the importance of the freshwater ecosystems, the anthropogenic pressures decrease water quality and affect the ecological health of river basins. The Water Framework Directive has the key goal of protecting the aquatic environment in its entirety, and establishes the importance of defining the ecological status of rivers with an interdisciplinary and multiparametric approach (2000/60/CE). In this study, we propose an integrated and holistic methodology to evaluate the river's health status taking into account chemical, ecological and ecotoxicological parameters. We used the Elsa River as a case study. It is a tributary of the Arno River, in Tuscany (Italy). The area is characterised by agricultural and industrial activities, by the presence of some cities and a river park. Chemical parameters of water (concentrations of nitrates, phosphates, ammonium and pH) were measured thanks to a citizen science project, inscribed to "Fresh Water Watch", carried on by the population and high schools of the area. For the ecological analysis, the EBI (Extended Biotic Index) and the FFI (Fluvial Functionally Index) were evaluated. The freshwater fish Italian chubs (*Squalius squalus*) were used as bioindicators, for the chemical and ecotoxicological analyses. We evaluated the microplastics ingestion, heavy metals and OCs concentration and a battery of biomarkers (AChE, EROD, PAHs metabolites, LPO, GST, LDH, IDH and ENA Assay). The results showed a general negative trend going from upstream to downstream, with some sources of macronutrients and contaminants, mainly in correspondence with the presence of inhabited centres. The ecotoxicological analysis showed a genotoxic effect correlated to Hg, PCBs and B(a)P metabolites in bile. The contamination is probably due to agricultural and industrial activities, vehicular traffic, polluted tributaries and inappropriate disposal of waste. The greatest abundance of microplastics was found in chubs caught in the river park, due to the presence of the urban area, the regular release of wastes along the river in the past, and the increase of tourism in the last years. This holistic approach has permitted us to define the health status of the river and identify the contamination and the sources of contaminants. This tool is helpful in promoting mitigation actions to block the sources of impact and bring the river to a good ecological state.

### 3.06.T-03 Temporal and Spatial patterns of Xenobiotics in German fish

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The complexity of the occurrence of emerging contaminants in aquatic environments is remarkable. The different physicochemical properties of the compounds and thus, their degradation and bioaccumulation processes affecting aquatic organisms are often missing due to a lack of analytical procedures to measure emerging compounds in biota. Moreover, the temporal trends in the occurrence of compounds that have been overlooked in the monitoring programs since decades are a scientific gap that could be tackled thanks to the sample repositories stored in the specimen banks across Europe.

The objective of this study is to apply the recently optimized protocol in the Plentzia Marine Station (EHU/UPV) to analyze xenobiotics in a biological tissue (fish filet) from a non-target point of view in historical and present biota samples.<sup>1</sup> A collection provided by the German Specimen Bank of 24 samples of bream from the Saale and Mulde rivers gathered biannually from 1996 to 2018, plus 25 samples from other rivers in Germany, including Rhine, Elba, Danube, and Saar among others, taken in 2018 was analyzed. The assessment was performed on a Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific™ Q Exactive™ Focus quadrupole-Orbitrap equipped with a heated ESI source (Thermo, CA, USA). Analysis of the extracts was performed in positive and negative mode in the Full scan data-dependent MS2 discovery acquisition. A selection of circa 250 compounds, including personal care products and pharmaceuticals, consumption products, industrial compounds (PFAS, plasticizers), and pesticides was used for the target analysis. Finally, a full suspect analysis with a database of more than 40,000 compounds from the NORMAN list will be presented.

Preliminary results in the targeted analysis revealed that 87 out of 304 emerging pollutants were present in the fish. Pesticides, parabens and phthalates were the families found at higher concentrations, but there were changes in relative presence through

the years and locations. For that, the assessment of archived biological samples is of great interest to perform retrospective analysis with new high-throughput analytical techniques.

1. Musatadi, M.; González-Gaya, B.; Irazola, M.; Prieto, A.; Etxebarria, N.; Olivares, M.; Zuloaga, O. Focused Ultrasound-Based Extraction for Target Analysis and Suspect Screening of Organic Xenobiotics in Fish Muscle. *STOTEN* **2020**, *740*, 139894. <https://doi.org/10.1016/j.scitotenv.2020.139894>.

### **3.06.T-04 Exploring Plant Protection Product Dynamics in Terrestrial Ecosystems: Insights from Bee Pollen**

*Sergio Cirelli<sup>1</sup>, Emmanuel Schaad<sup>1</sup>, Christina Kast<sup>2</sup>, Thomas D. Bucheli<sup>3</sup> and Aurea C. Hernández<sup>1</sup>, (1)University of Bern, Switzerland, (2)AgroSwiss Bee Research Centre, Agroscope, Switzerland, (3)Environmental Analytics, Agroscope, Switzerland* Plant protection product (PPP) interactions with the environment are still challenging to assess because of their multiple input sources and intricate ecosystem dynamics. Biomonitoring techniques have emerged as a promising tool, offering valuable insights into the processes and factors influencing PPP fate and transport. In this study, we utilize bee pollen from *Apis mellifera* as a tool for terrestrial PPP monitoring in an area covering 39 fields cultivated according to standard agricultural practices. Our research integrates chemical analysis of pollen collected during cultivation season with an extensive dataset on PPP applications. This dataset includes details on applied substances, quantities, application timing, and weather conditions, forming a robust foundation for our analysis. It offers valuable insights into the terrestrial exposure of pollinators, enabling a more detailed evaluation of PPP dynamics.

In brief, we collected weekly pollen samples from April to early October from five beehives strategically placed adjacent to a meteorological station. We pooled the hive samples each week to represent the bee population comprehensively. Additionally, we isolated specific fractions through targeted pollen fractionation, enhancing our ability to discern contamination patterns and identify sources. The pollen analysis was performed by target and non-target analysis using liquid chromatography coupled with mass spectrometry (LC-MS/MS) and high-resolution mass spectrometry (HRMS) to explore unknown substances by tracking their presence based on concentration trends.

Our results reveal a substantial presence of PPP contamination in pollen samples, with 50% of identified compounds aligning with those applied in our controlled area. Further interpretation of the detailed PPP application allowed us to pinpoint the specific pathways and factors influencing PPP contamination in the pollen. Moreover, our analysis of pollen fractions shows that wildflowers are the primary source of PPP in pollen. This result highlights a significant non-target species pathway through which these contaminants enter the pollinator ecosystem.

In summary, this study presents a comprehensive exploration of PPP contamination in bee pollen, providing unique insights into environmental dynamics and highlighting the suitability of this matrix as a biomonitoring tool for terrestrial ecosystems.

### **3.06.T-05 Polyhalogenated Carbazoles in the Food Web of the St. Lawrence Estuary Beluga Population.**

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Contaminants represent a major stressor that may adversely affect the health and recovery of the endangered St. Lawrence Estuary (SLE) beluga (*Delphinapterus leucas*) population (Canada). However, the exposure of SLE belugas to many emerging contaminants remains unknown. Such emerging contaminants of concern are the Polyhalogenated Carbazoles (PHCZs), which are impurities in halogenated indigo dye formulations or carbazole-containing polymers that are used in electronic devices. PHCZs are structurally similar to halogenated dibenzofurans, and can pose dioxin-like toxicities to organisms, and are known to bioaccumulate and biomagnify in food webs. Some PHCZs have previously been reported in sediments and fish of the Great Lakes of North America, but their levels in the SLE, which is located downstream of the Great Lakes and home to SLE beluga, are unknown. To fill this knowledge gap, tissues were collected from SLE beluga (n=7 including liver and blubber) and 17 known and potential prey (n=183 including 15 fish species and 2 invertebrates) of SLE beluga in 2019 and 2020 to investigate the occurrence and trophodynamics of 11 PHCZs in the SLE beluga's food web. Tissue samples were analyzed for nitrogen isotopes ratios ( $\delta^{15}\text{N}$ ) to assess trophic position. All target PHCZs were detected in prey samples except for 1,3,6,8-tetrabromocarbazole (BCZ-1368). Concentrations of total PHCZs in prey samples were in the range of  $2.3 \pm 0.3$  to  $50.7 \pm 19.1$  ng/g (mean  $\pm$  SE) (lipid weight (lw)), thus lower than previously reported concentrations in fish from the Great Lakes ( $66.1 \pm 20.3$ - $181 \pm 141$  ng/g (lw)). The trophodynamics of target contaminants with 100% detection indicate that 36-dichlorocarbazole (CCZ-36) was positively correlated to  $\delta^{15}\text{N}$ , indicating biomagnification in the SLE food web. The estimated toxic equivalent (TEQ) concentration of total PHCZs for the analyzed species (mean range: 0.20-1.62 pg/g lw) was lower than for fish from the Great Lakes (8.7-25.7 pg/g lw) and San Francisco Bay (2.5-34.8 pg/g lw), and harbor seals from San Francisco Bay (7.6-174 pg/g lw). This study provides a baseline for monitoring PHCZs in the SLE beluga food web, and enhance our understanding of the mechanisms underlying the biomagnification of PHCZs in aquatic food webs.

### 3.06.P Application of Biomonitoring Approaches to Support Surveillance of Chemical Exposure in the Environment

#### 3.06.P-Mo114 Exposure contribution and integrated exposure assessment of methyl paraben and propyl paraben

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Parabens, including methyl paraben (MP), ethyl paraben, and propyl paraben (PP), are widely used as preservatives in cosmetics, stored foods, beverages, and personal care products to inhibit the growth of microorganisms. Previous studies mainly focused on one exposure source and specific exposure route to evaluate external exposure, and limited information is available on various exposure sources and routes of parabens. In this study, a library of exposure sources and contamination data for MP and PP was collected through a literature review, and the contribution of paraben exposure was evaluated across different life stages, including infants, preschool children, school-age children, adolescents, and adults. In addition to the conventional sources of exposure, pharmaceutical intake scenarios were established for the infants and children population. Although data on PP contamination levels are limited compared to MP, both parabens contribute significantly to exposure through oral and dermal routes. The oral exposure contribution rate was high in the order of fruits, vegetables, and paste in children, adolescents, and adults, however, infants had a higher exposure contribution through breast milk in both parabens. For dermal exposure route, body/hair products, cleansing products, skin care products and color cosmetics were identified as major sources of MP. The dermal exposure source of PP shows comparable results, with the exposure levels from oral care products being higher than those of methyl paraben. The contribution rate of PP was found to be high from consuming sources, inhaling indoor air, and using cleansing products. The results of this study can be used for integrated exposure assessments of other parabens, and emphasize the need for ongoing monitoring of MP and PP in various products.

Keywords: Exposure assessment, Alternative phthalates, Intervention study

#### 3.06.P-Mo115 Legacy and Emerging POPs in a Rare Greenland Shark Stranding

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Greenland sharks (*Somniosus microcephalus*) inhabit cold, deep Arctic and North Atlantic waters and they are thought to be the longest-lived vertebrate on Earth; potentially living for 400 years. Growing only one cm/year up to five metres in length, this species is considered vulnerable due to a combination of its long maturation period and low fecundity, as well as facing pressure from pollution and overfishing. Further, this species is considered an apex predator in Arctic food webs, feeding on a variety of dead and live fish, squid and mammals. Sightings/strandings of this shark in the UK are extremely rare, however a recent stranding on the coast of Cornwall in 2022 presents a unique opportunity to learn more and monitor this species. By studying the contaminant load of this shark, we can seek to understand how anthropogenic pollutants impact marine biota. This is particularly pertinent given previous declines in biodiversity linked to the input of organic chemicals into the oceans. Here, we present data on the organic contaminant loading measured in a Greenland shark sample, including PCBs and PFAS compounds. We used liver tissue to study these contaminants and followed established extraction and clean-up procedures for each pollutant group, using GC-MS and LC-HRMS to analyse the samples. Several PCB congeners, DDTs and PFAS compounds were detected in the shark at measurable concentrations. Pollutant burdens were dominated by PFAS compounds, which mainly consisted of long-chain PFCAs, similar to other shark species. DDTs were the second most dominant pollutant, followed by PCBs with hexa- and hepta- congeners accounting for >50% of the PCB burden. Levels of PCBs were generally lower than those reported for other large-bodied elasmobranchs and for Greenland sharks stranded in their typical home range, whereas PFAS concentrations were found to be comparable or even higher. Our research addresses the need to understand exposure levels in a lesser studied species to both legacy and emerging contaminants.

#### 3.06.P-Mo116 Development of biomarkers for immunotoxicity in mussels: an in situ experiment

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The European Union has adopted the Marine Strategy Framework Directive (MSFD) to protect and restore the marine environment and achieve a good environmental status (GES). The MSFD defines 11 descriptors of GES regarding biodiversity, food webs, seafloor integrity and *contaminants*. Biomonitoring is a crucial tool to preserve good environmental status in the marine environment. New chemicals are entering the market every day and our consumption of chemicals is still increasing. The receiving end of this is the water systems that will eventually lead to the marine environment. In our toolbox for biomonitoring there are several biological effects markers (biomarkers) that can help in assessing the exposure of pollutants. These biomarkers can report on disturbances in several physiological systems, such as reproduction and toxicant metabolism. But there is still a lack of biological effects markers that can be used to indicate immunotoxic effects in marine organisms. This is due to the complexity of the immune system, lack of information provided in papers and difficulties in linking chemical mode of action to immunological effects in organisms.

In order to suggest new such biomarkers the current project has the aim to optimize and develop biomarkers for immunotoxicity in fish and mussel that can be used in annual biomonitoring in Sweden and the rest of the European Union.



Fish and mussel were sampled from a known polluted marine site and from a reference site with pristine waters. *Zoarcas viviparous* and *Mytilus edulis* were chosen since they are stationary organisms and can thus reflect the effects in the local environment. In the laboratory the two species were exposed to bacterial LPS for three days to trigger the immune system. After the three days they are euthanised and candidate biological effect markers were analysed. The markers were selected through literature search and previous research in our laboratory. Several of these markers has shown promising results and will be further developed in this novel experimental setup. The biomarkers that were analysed are cytokines, complement system activity, lysozyme activity, IgM concentration, c-reactive protein, white blood cells/haemocyte count, leukocyte viability, phagocytic capacity, and gene expression of relevant genes such as Diablo. Results on how the capacity of these parameters respond to a pathogen insult is affected by long time exposure to toxicants will be presented.

### **3.06.P-Mo117 Using the Otter and Buzzard as Sentinels for the Assessment of Spatial and Temporal Trends in Synthetic Chemical Pollution between Contiguous Aquatic and Terrestrial Environments**

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Synthetic chemicals are pervasive in our environment, and the exposure of terrestrial and freshwater biota to these compounds presents a growing concern. Conventional monitoring primarily relies on targeted screening of abiotic samples, potentially missing toxicologically relevant chemicals that exist at concentrations below detection limits in such samples. Some chemicals may accumulate in biota, making their detection achievable even when undetectable in abiotic samples. Additionally, wide-ranging biota can serve as integrators of temporally and spatially heterogeneous chemical inputs, offering a more comprehensive perspective.

The study explored differences in chemical occurrence, concentration, and temporal trends, between otters (*Lutra lutra*) and buzzards (*Buteo buteo*) in the UK. We aimed to evaluate whether differences in chemical pollution between the two top predator species might indicate differences between aquatic and terrestrial environments.

Otter and buzzard carcasses were collected from a common geographical zone within the UK between 2002 and 2019. Liver samples from both species were analysed through the LIFE APEX programme in LC-QToF-MS wide-scope target analysis of over 2,200 contaminants, which spread over a broad spectrum of applications and uses. Statistical analysis and modelling were conducted to provide quantitative results.

We highlight the efficacy of the chosen sentinels for monitoring chemical pollution and describe the wide range of synthetic chemicals in both the terrestrial and freshwater environment. Comparisons highlight the differences in both occurrence and concentration between the two compartments, which confirms that the inclusion of both sentinel species is beneficial in understanding environmental chemical pathways, and thus shows the species to be a valuable resource for pollution surveillance over both space and time. The insights gained from this study therefore contribute to the development of more robust biomonitoring strategies, which are more effective for evaluating the risks associated with synthetic chemicals to both wildlife and human health.

### **3.06.P-Mo118 Active Biomonitoring of River Pollution Using an Ex-Situ Exposure System With Model Species.**

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In the last two decades, the evaluation of environmental quality has grown in importance, leading to the necessity to develop new methods to better integrate contamination and its impact on aquatic organisms. Sensitivity of early life stages of fish to contamination makes them especially valuable for monitoring applications. However, their development is highly influenced by water parameters (such as temperature) which poses a challenge to their use in active biomonitoring.

The *ex-situ* exposure method involves a direct and continuous exposure to river water flow but in controlled temperature, oxygen and photoperiod conditions. The control of environmental parameters allows to eliminate the potential influence of these confounding factors on the results interpretation. This method has been used in spring 2023 on the Garonne river (France) to conduct Fish Early-Life Stage Toxicity Tests (OECD TG 210) using Japanese medaka (*Oryzias latipes*) embryos. They were exposed to river water throughout their development from 24h post-fertilization up to 2 days after hatching, while temperature and oxygen saturation were controlled. Mortality during embryonic development was significantly higher for those exposed to the Garonne water compared to control in the two exposure series conducted, with the highest rate of mortality occurring during organogenesis..

In parallel, experiments on the freshwater amphipod *Gammarus fossarum* have been conducted also by using the *ex-situ* exposure. This species is widely used in active biomonitoring and standardized tests exist in France for this purpose. Reprotoxicity and feeding rate were assessed while controlling water parameters. The results did not reveal a significant

reproductive toxicity on the organisms. However, an induction of feeding rate was observed in gammarids exposed to the Garonne water compared to control in certain batches.

Water contamination was also assessed. Passive samplers were deployed to monitor pesticides and samples of water were taken to quantify metal concentrations in water. Results will be available and presented in May 2024.

These results highlight the significance of the exposure method, particularly in controlling water parameters to expose more sensitive organisms and reveal substantial variations in the toxicity of the Garonne River's water depending on the exposed taxon.

### **3.06.P-Mo119 Method development and application for simultaneous determination of hydroxy derivatives of PAHs in wastewater for using in wastewater-based epidemiology**

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It is important to assess the exposure of PAHs, especially in urban areas. Exposure to PAHs causes a number of negative health effects, including: reproductive defects, DNA mutations, leukemia and cancer of the lung, bladder, bone, brain and scrotum. There is a lack of knowledge and understanding of the local exposure to organic air pollutants, especially in highly urbanized regions of Eastern Europe. PAHs entering the body in a certain part are removed in unchanged form, but usually undergo several steps of biotransformation and detoxication process and are excreted as hydroxymetabolites through urine and feces.

The aim of the study was the determination of the hydroxyl PAH metabolites profile in wastewater. The 24 composite samples of wastewater influent were collected at the "Plaszow" wastewater treatment plant (WWTP) in Krakow, in winter and summer. This plant is the biggest one in the city and the third in the country, treating over a 70% of Krakow wastewater with an average capacity of 165.000 m<sup>3</sup>/day.

Studies were carried out using SPE-GC-MS/MS method for the determination of selected OH-PAH (1-hydroxy and 2-hydroxynaphthalene (1-OH-NAP, 2-OH-NAP), 2-hydroxy and 9-hydroxyfluorene (2-OH-FLU, 9-OH-FLU), 9-hydroxyphenanthrene (9-OH-PHEN), 1-hydroxypyrene (1-OH-PYR), 3-hydroxybenzo(a)pyrene (3-OH-BaP)) in samples collected in WWTP. Analysis was carried out by means of a Thermo Scientific GC TRACE 1300 (GC-(IT)MS/MS) and a TriPlus RSH Autosampler.

The average concentrations of compounds ranged from 5 ng/L to more than 400 ng/L. The highest concentrations of OH-PAH were detected in the influents, for 2-OH-NAP and 9-OH-FLU in winter and summer. However, their concentrations were twice lower in summer. A similar trend was observed for other compounds. 1-OH-PYR was observed for influent and effluent samples only in winter at average concentrations of 8 ng/L and 5 ng/L, respectively.

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### **3.06.P-Mo120 Methods Enabling Accurate Data on Microplastics Abundance and Distribution: Applicability for Biomonitoring Programs**

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The presence of microplastics (MPs) in marine ecosystems is of considerable concern. In recent years, many effort has been made to quantify MPs pollution in marine biota using a wide variety of approaches. One of the main problems in the visual quantification of MPs is the overestimation. For a realistic assessment of MPs in the environment chemical analysis such as Raman spectroscopy are needed which allow to accurately identify the nature of the particles observed under the stereoscope in monitoring programs. Thus, the aim of the study was (1) to define the most accurate methodology for the identification/quantification of MPs in environmental samples and (2) to provide precise data of MPs abundances in mussels from the Urdaibai Biosphere Reserve (case study). Mussels *Mytilus galloprovincialis* were collected seasonally during a one-year monitoring program in two sampling points (TXA and TE) along the Urdaibai estuary. Samples were alkaline digested with 10% KOH (40° C during 48 h) and filtrated through a vacuum filtration system using 30 µm metal filters. Filters were first analysed visually by stereoscope and then with the aid of Raman. For the identification of MPs in Raman, a grid overlay system has been developed to localise visually detected MPs. After visual observation the amount of MPs was much higher than after Raman confirmation, with an overestimation of 88% of MPs. Confirmed fibers and fragments were detected in mussels from both sites, being fibers predominant. The MPs accumulated by the mussels from TXA were PET, PE, PP and PS; while in TE PET and PE were identified. Blue was the predominant color observed in MPs ingested by mussels in Urdaibai.

The results show differences in the abundance of MPs between seasons for TE, with a significant decrease and increase in February and April, respectively. Moreover, significant differences were observed between sampling points in February and April. Based on these results, Raman spectroscopy is crucial to recognize environmental MPs with high reliability, which enable a reliable diagnostic and assessment on MPs occurrence and fate in marine ecosystems. Funded: Basque Government (IT1743-22, IT- 1446-22) and MICINN (PID2020-118685RB-I00).

### **3.06.P-Mo121 Concentrations of Mercury in Water, Sediment and Blood of Grass Carp and Their Associations with Oxidative Stress Biomarkers**

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Mercury (Hg) is a toxic metal that can accumulate in aquatic ecosystems and pose a risk to human and wildlife health. Mercury in fish blood can cause oxidative stress, which is a condition where the production of reactive oxygen species (ROS) exceeds the capacity of the antioxidant system to neutralize them. ROS can damage cellular components such as lipids, proteins, and DNA, and impair the normal functioning of cells and tissues. The aim of this study was to investigate the concentrations of Hg in water, sediment, and whole blood of grass carp from a university experimental freshwater fishpond in Slovakia, and to examine their associations with biomarkers of oxidative stress. Mercury in fish blood and oxidative stress are closely related and can be used as indicators of mercury toxicity in fish. 20 samples were obtained from each matrix. Total Hg was measured by cold vapor atomic absorption spectrometry. Biomarkers of oxidative stress (reactive oxygen species, glutathione peroxidase, and lipid peroxidation) were assessed in grass carp blood samples. The highest concentrations were observed in sediment samples, followed by whole blood samples, and the lowest concentrations were in water samples. Hg concentrations in carp blood were positively correlated with biomarkers of oxidative stress (ROS, GPx and MDA), but without significant differences. Hg levels in water were statistically significantly negatively correlated with GPx and total weight of fish. Analysis of biomarkers indicated a negative association between GPx and ROS, and GPx and MDA. These findings suggest that carp fish from the lake are exposed to low levels of Hg, but slightly show signs of Hg-induced effect in their blood and provide insight into oxidative stress defences in grass carp. Further studies are needed to evaluate the potential health effects of Hg exposure on carp fish and other aquatic organisms in the lake. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-21-0168. This publication was also supported by The Ministry of Education, Science, Research and Sport of the Slovak Republic under the project VEGA 1/0571/23.

### **3.06.P-Mo122 Wastewater Micropollutant Surveillance Using Effect-based Biomonitoring**

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As availability of water resources decreases, the apparition of new contaminants of concern (CEC) identified in water networks continues to increase. Current wastewater monitoring in treatment plants relies on interval based grab sampling measurements for a select few chemicals or for punctual effluent toxicity assessment using a restricted choice of bio-assays. As this strategy is known to omit a portion of micro-pollutants and contaminants of emerging concern (CECs), innovative approaches to wastewater management are essential to attempt to avoid potentially detrimental impact of largely unknown CEC discharge in aquatic ecosystems and improve efficiency of urban water re-use.

In previous work using the biomonitoring apparatus ToxMate, whereby the video-tracking of locomotor behaviour of bio-indicator invertebrates (*Gammarus*, *Radix*, and *Erpobdella*) generates positional data in the on-line wastewater multi-species monitoring device, we showed the biomonitoring method is an effective in non-targeted effect-based screening of micro-pollutant presence (including little known emerging contaminants). Our current research illustrates results from lab studies and long term surveillance in WWTPs, where the characterization of the effluents in real-time for the multispecies approach alerts operators and allows strategic sampling, giving a better understanding of effluent composition. Laboratory testing has led to the accumulation of behavioural reaction analysis for over 80 micropollutants, allowing the definition of multi-species behavioural fingerprints to characterise causes for temporal variability in CEC discharge, presented using functional data analysis (FDA) and artificial intelligence. Fingerprints are then used to link alerts to their potential chemical nature.

Continuous surveillance not only identifies critical moments but has incorporated timely sampling techniques to validate reactions. Couple with behavioural fingerprinting, these moments may help adapt wastewater management strategy in real-time. Thus, the potential is not only limited to the improvement of aquatic environmental quality through wastewater management, but could also provide concrete indicators of the suitability of re-use in various domains such as urban, agricultural and leisure use.

### **3.06.P-Mo123 Assessment of Temporal Trends in Quality of European Surface Waters: Towards a New Biological Indicator**

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The European Topic Centre Biodiversity and Ecosystems (<https://www.eionet.europa.eu/etcs/etc-be>) is a Consortium of 24 organisations led by the Norwegian Institute for Water Research (NIVA). The consortium is working in partnership with

the European Environment Agency (EEA) under a Framework Partnership Agreement for the period 2023-2026. The ETC BE contributes to EEA's integrated systemic assessments of terrestrial, freshwater and marine ecosystems. These assessments include effects of climate change and how to mitigate climate change, cumulative drivers and pressures on these ecosystems, as well as socio-economic implications of solutions. Assessments of spatial and temporal trends in the aquatic environments across Europe are fundamental for these assessments and are based on a set of indicators such as «Industrial pollutants released to water», «Pesticides in rivers, lakes and groundwater», «Nitrate in groundwater and rivers», and «Nutrients in transitional, coastal and marine waters in Europe». EEA indicators are designed to answer key policy questions and to support environmental policy making from policy monitoring and evaluation to communicating to policymakers and the public.

Here we focus on a new candidate indicator, «Biology in rivers, lakes, transitional and coastal waters». This indicator is based on ecological quality ratios (EQR), which are normalised in a continuous scale 0-1, and therefore more informative than the 5-level ecological status class data reported to the Water Framework Directive. EQR values have been reported to EEA from more than 20 European countries through the WISE-2 Biology data flow within WISE-SoE (Water Information System for Europe – State of the Environment); mostly from river and lakes, but also from transitional and coastal waters. The EQR values represent the impacts of specific pressures such as nutrient enrichment and organic pollution, hydromorphological pressures or acidification, and can therefore provide a link to physical and chemical stressors affecting the ecological status of water bodies. The use of normalised EQR values enables both analysis of geographical and temporal patterns, statistical trend analysis, and quantitative relationship with physical/chemical stressors. However, the reported WISE-2 Biology data still show variations in the number of water bodies and years among countries, which represent challenges concerning its representativity as a European-scale indicator.

### **3.06.P-Mo124 Challenges and Opportunities of Building a Collaborative Chemicals Biomonitoring Framework Today, for the Chemicals of Tomorrow**

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A proposed collaborative terrestrial chemicals biomonitoring framework for England will be presented. The aim of the framework is to produce impactful 'fit for purpose' data and evidence for use in environmental, chemical and agrochemicals, regulation and policy, and conservation delivery. An overview of the terrestrial chemicals monitoring in England will be presented alongside, developmental terrestrial indicators of exposure and effects on wildlife, reported on against available toxicity thresholds over time.

The key components of the terrestrial biomonitoring framework will be shared. This will include the process and scientific investigations undertaken, in selecting sentinel species and analysis of new and archived samples for contaminant residues, as well as the application of systems thinking to develop sample and data platforms and protocols. Technical challenges will be discussed with a focus on development of tissue sample and data archives, sampling design, and decision-making tools used to deliver the data needs of today while also being future proof. Attention will be drawn to the need for monitoring data to improve chemical prioritisation and early warning systems in the terrestrial environment. In addition to the need to build a data architecture with clear and robust data ethics principles and guidance, that enable the delivery, housing, use, and sharing of non-targeted screening data.

### **3.06.P-Mo125 Temporal Trends of Dietary Patterns and Mercury in Norwegian Killer Whales (*Orcinus orca*) from Contrasting Ecosystem States**

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Killer whales are long-lived, occupy a high trophic position, and are valuable bioindicators to assess marine ecosystem health and exposure to persistent contaminants. Norwegian killer whales were formerly categorized as fish specialists strongly associated with Norwegian Spring Spawning Herring (*Clupea harengus*) (NSSH), but are now known to incorporate other prey, including marine mammals, into their diet. The NSSH stock collapsed in 1970 due to overfishing but have since recovered. This study aimed to evaluate historic and contemporary dietary variations possibly correlated to changes in preferred prey availability, and to assess changes in mercury (Hg) concentrations between and within individuals over time by (1) investigating annual patterns of inter and intraindividual dietary trends and Hg accumulation over both time periods, (2) evaluating whether shifts in preferred prey resources caused by fishing pressures played a role in killer whales integrating more marine mammals in their diet, (3) determining if feeding on higher trophic prey resulted in a greater body burden of Hg, and (4) to identify temporal trends of Hg intake in response to changes in emissions.

Bulk stable isotopes of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ), and Hg concentrations were analysed from annual growth layers of killer whale teeth (n=21) from two groups living in contrasting ecosystem states. Teeth were sectioned longitudinally with a

diamond saw and two slices (circa. 1 mm width) were cut from each half. The left slice was ground into a powder mixing all GLGs, and the right piece was decalcified in a 0.25 M HCl solution. Each growth layer was extracted and minced using a scalpel. Samples were prepared for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis performed by the Institute for Energy Technology (Kjeller, Norway). Mercury analysis is scheduled for December 2023 at the University of Oslo.

Preliminary results show the contemporary group exhibits higher  $\delta^{15}\text{N}$  values and a greater range than the historical set. This could indicate a higher variability in dietary patterns and a larger consumption of marine mammal prey.  $\delta^{15}\text{N}$  values for powdered tooth samples representing all combined GLGs were greater for the contemporary set ( $\delta^{15}\text{N}$ : Max=15.59 ‰, Min=11.82 ‰) than the historical samples ( $\delta^{15}\text{N}$ : Max=13.14 ‰,  $\delta^{15}\text{N}$  Min=11.40 ‰). This project offers six decades of insight on how Hg has been accumulating in wildlife pre- and post-emission reductions brought forth by the Minamata Convention.

### **3.06.P-Mo126 Occupational (fishermen and recycler workers) exposure assessment to organophosphate esters, phthalate esters, and alternative plasticizers through human hair analysis**

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Due to their widespread use in consumer products, organophosphate esters (OPEs) and phthalate esters (PAEs) are ubiquitous in the environment, leading to potential human exposure through ingestion, inhalation, and dermal absorption. Both OPEs and PAEs are used as plasticizers, and some specific OPEs are also used as flame retardants (FR). They are mainly used in plastic materials, such as household appliances, furniture, textiles and personal care products. OPEs consumption was increased to be used as alternatives to polybrominated diphenyl ethers (PBDEs), which were FRs banned in the Stockholm Convention on Persistent Organic Pollutants. Hair samples were collected from volunteers of two occupational groups: a) recycler workers (n=32) at industrial recycling park in Cartagena city (Colombia) and b) fishermen (n=36) in local communities across of Bolivar state (Colombia) as well as their families. Extraction was done by pressurized liquid extraction (PLE) using an ASE 350 system (Dionex, Sunnyvale, CA, USA), with hexane:acetone (1:1) as solvent extraction and working at 1600 psi and 50 °C. After PLE, extracts were concentrated to incipient dryness and re dissolved with methanol for a final volume of 500  $\mu\text{L}$ . The extracts were injected in an online sample purification and analysis method based on turbulent flow chromatography (TFC) (Thermo Scientific TurboFlow™ system) coupled to a triple quadrupole (QqQ) tandem mass spectrometry (MS/MS) and a heated electrospray ionization source (HESI) was used. The most intense transition was used for quantification, while the second provided confirmation. Concentration levels of 16 OPEs, 11 PAEs and 4 alternative plasticizers have been measured in hair samples from recyclers and fishermen in Colombia, which shows the occupational exposure to these compounds. In the case of OPEs, it has been seen that recyclers presented higher concentration levels in hair than the fishermen, while for PAEs and their alternatives there were not found differences in concentrations. This study demonstrates the potential of using human hair as a biomarker for assessing exposure to PAEs and OPEs, particularly in occupational settings, with a non invasive sample collection and a rapid analysis. The findings highlight the need for improved safety measures and regulations to reduce PAE and OPE exposures in high risk occupations

### **3.06.P-Mo127 Environmental Exposure to Anticoagulant Rodenticides and $\alpha$ -Chloralose in the Liver of Domestic Cats (*Felis catus*) in Slovenia**

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Due to their mass and continuous use, rodenticides pose a significant environmental problem as they can have harmful effects on non-target wildlife and domestic animals. For this reason, the residues of anticoagulant rodenticides (ARs) and an alternative rodenticide  $\alpha$ -chloralose were monitored in domestic cats (*Felis catus*) in Slovenia. In the period from 2021 to 2022, 99 free-roaming or partially free-roaming cats were collected from veterinary clinics in all geographical regions of Slovenia. Their livers were extracted with methanol/water (2:1, v/v), cleaned-up using a solid supported liquid-liquid extraction and measured by liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS/MS) with reporting limits in the range of 0.2 – 10.0 ng/g. Residues of at least one substance investigated were detected in 64.7% of the analyzed livers. Concentrations of pooled ARs ranged from 0.8 to 1,819.7 ng/g with a mean and median value of 110.8 and 22.9 ng/g, respectively. The second generation ARs (SGARs) brodifacoum, bromadiolone, difenacoum and difethialone were found in 53.5, 25.3, 8.1 and 2.0% of the samples, respectively. Flocoumafen was not detected. We determined brodifacoum at concentrations of  $\geq 800$  ng/g in 3 of 99 cat livers (3.0%) and one of these (1%) contained residues of  $>1,800$  ng/g. Coumatetralyl was mainly found in the first generation of ARs (FGARs) and was determined in 21.2% of the samples, all of which were below 4.1 ng/g, while warfarin was found in one sample (1.0%) with a concentration of 14.1 ng/g and chlorophacinone was not detected.  $\alpha$ -chloralose was found in one sample (1.0%) with a concentration of 561.7 ng/g. Two older animals living outdoors contained hepatic residues of as many as 4 substances (three SGARs and one FGAR). As the results of the study indicate a potential toxicological risk for domestic cats in Slovenia, asymptomatic poisoning related to rodenticides in this species should be continuously monitored in the future. The relevance of the study lies in its complementarity to the assessment of the environmental risk of rodenticides to non-target animals in the Western Balkans. The results highlight a

potential environmental problem related to the persistence, bioaccumulation, secondary poisoning and toxicity of rodenticides in this region and will contribute to the knowledge of the protection of European ecosystems.

### **3.06.P-Mo128 Determination of the presence of endocrine disruptors in breast milk samples from the municipality of Malambo/ Atlántico (Colombia)**

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Background: Breast milk has an important role in the first month of life, because in this phase there are a series of changes in the neonatal organism and important adaptive mechanisms are developed in the child's relationship. Breast milk has been used as a biological marker of environmental pollution due to bioaccumulation processes in fatty tissue; many chemical compounds reach easily measurable concentrations. Endocrine disrupting chemicals (EDCs) are compounds capable of affecting the normal balance of hormonal functions in animals and their progeny and can pass into breast milk, becoming a possible threat to newborns. OBJECTIVE: determine levels of EDCs in breast milk samples from the municipality of Malambo/Atlántico. METHODOLOGY: The levels of EDCs in breast milk were measured by IR spectroscopy. RESULTS: breast milk samples from 25 donors were analyzed, mainly finding products that are used in industrial applications, specifically in the field of plastics and polymer such as: acrylamides, pentaerythritol, aminocarbonyl ethene, 2-Butene 1,4-Diol CONCLUSION: it is necessary to identify the substances that may be affecting the quality of breast milk in the different stages of maturation and in this way evaluate the consequences on the health of breastfeeding children.

### **3.06.P-Mo129 Plane tree leaves: An integrative bioindicator for monitoring air concentrations of Semi Volatile Organic Compounds**

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The use of plant species to assess air contamination is now well reported. Indices of overall air quality have been established, for example, with lichens that can accumulate certain contaminants, in particular, metallic trace elements. Higher plant species also show an accumulation of some organic compounds (PCBs, PAHs) in the leaves via absorption in the waxes of these contaminants present in the gaseous phase of the air. Here, the studied model is the plane tree leaf for its ubiquitous presence in contrasting environments and its long leaf period allowing a greater accumulation. A global procedure, from sampling to analysis, was validated with leaves of plane trees to quantify 6 families of semi-volatile organic compounds (SVOCs): Phthalates, PAHs, PCBs, Organochlorine Pesticides, PBDEs, and Parabens. The leaves are harvested at the end of the autumn and then washed, dried, crushed and extracted by an Accelerated Solvent Extraction system (ASE). The extract is split into 3 on a cartridge of Florisil. The fractions are then purified prior to analysis in GC/MS, GC-MS/MS or LC-MS/MS. The quantification limits are in the order of ng/g except for the phthalates (tens of ng/g). Plane tree leaves were sampled in the region of Paris (France) and during 2 periods (2002 and 2012-14).

- The contents in the leaves and the concentrations in the gaseous phase of the air (active sampling) show a good correlation.
- At the spatial level: differences are observed between several contrasting sites: forest, rural, urban, dense urban, traffic.
- At a finer spatial scale, two contrasting urban sites (distance < 1.3 km) also show an influence of traffic on PAHs levels in the leaves.
- At the temporal level: at the same urban site, Paris center, the harvested leaves with a 10-year difference show a variation of air contamination. A significant decrease in leaf levels is observed: from 5 to 10 times, depending on the families studied, except for parabens where a slight increase is obtained. These concentrations are related to the practices and uses of the contaminants studied.

These results show:

- there is an accumulation of SVOCs in plane tree leaves in relation to the sources and uses of these contaminants.
- this tool is relevant to be used for the biomonitoring of the gaseous phase of the air.

### **3.06.P-Mo130 Urinary Chromium as Biomarker Among Lung Adenocarcinoma Patients in Vojvodina**

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Introduction: The occupational exposure to chromium is associated not only to increased incidence of lung and nasal cancers, the buccal cavity and the pharynx cancers but also to enhanced risk of overall mortality due to lung, larynx, bladder, kidney, testicular, bone, and thyroid cancer. Mechanisms of chromium toxicity and carcinogenicity are not completely understood since chromium (VI) is unreactive towards most biomolecules, the impact of Cr (VI) on the cellular stress response might had a central role in its adverse effects. Moreover, due to chromium intracellular reduction chemically versatile species are

generated and may attribute to its carcinogenicity. In this study, the urinary chromium levels among advanced lung adenocarcinoma male patients were determined.

**Methodology:** A total of 36 male patients (39-84 years old) with inoperable IIIB and IV stadium of lung adenocarcinoma, diagnosed in the Institute for Pulmonary Diseases of Vojvodina, Serbia were enrolled in the study. All participants provided their first morning urine samples in which chromium concentrations were determined by ICP-MS.

**Results:** In 38.99% (14/36) urine samples Cr was detected above the limit of quantification. The measured chromium levels were above urinary mean levels 0.22 µg/L for healthy adults defined by the ToxGuide of the US Agency for Toxic Substances and Disease Registry. The chromium urinary concentrations varied between 5.585 and 117.544 µg/L. The maximum urinary chromium level when dilution factor was taken into account was as high as 555.067 µg/g creatinine.

**Conclusion:** Almost 40% of lung adenocarcinoma patients (with inoperable IIIB and IV stadium) from Vojvodina were detected with high urinary chromium levels which are above mean urinary levels for healthy adults.

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### **3.06.P-Mo131 Heavy Metals as Risk Factors for Neuroendocrine Lung Cancer - Preliminary Results of Biomonitoring Study**

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Heavy metals such as Hg, Cd, Ni, Cr and Pb are ranked as the first class of hazard due to their high toxicity. The continuous exposure to heavy metals, even at trace levels, is associated with various biochemical and physiological alterations and serious health problems. Although the lung cancer incidence and mortality rates significantly differ among countries, this type of cancer is still the major cause of cancer associated death worldwide. Neuroendocrine cancer is reported in 2% of all lung tumors cases in adults as less-frequent and -invasive lung cancer. However, the role of environmental risk factors, such as heavy metals in onset and progression of neuroendocrine lung cancer is still unclear. Hence, the aim of this biomonitoring study was to investigate the load of heavy metals in patients with neuroendocrine lung cancer. In this preliminary study four men and six women (aged 57-76) with inoperable IIIB and IV stage lung neuroendocrine cancer were enrolled. The levels of heavy metals (Hg, Cd, Ni, Cr and Pb) were estimated in morning urine samples after mineral digestion using inductively coupled plasma mass spectrometry analysis (ICP-MS spectrometer 7700x, Agilent Technology, Germany). At least one heavy metal was quantified in urine above limit of quantification (LOQ) in both women and men. More than half (60%, 6/10) of neuroendocrine lung cancer patients were exposed to Cd and Hg. Although, nickel and chromium were measured above LOQ in smaller number of collected samples, the quantified levels (24.2-114 µg/L for nickel and 18.1-29.3 µg/L for chromium) were much above the recommended concentrations (1-3 µg/L for Ni and 0.22 µg/L for Cr). In addition to Cd and Hg, Pb was quantified at concentration 13.4 µg/L in one female sample. Despite the small sample size, the obtained results suggest that lung neuroendocrine cancer patients were exposed to mixture of heavy metals. Further studies are needed in order to better elucidate if high exposure to heavy metals might lead to the poor prognosis in lung cancer patients.

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### **3.06.P-Mo132 Evaluation of biomarkers of effect due to exposure to environmental fine particulate matter (PM 2.5) in a population neighboring an industrial park in Cartagena, Colombia**

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**Introduction:** Air pollution is the main environmental risk for human health. The World Health Organization (WHO) estimated that air pollution caused 4.2 million premature deaths in 2019 worldwide. Air pollution consists of a variable mixture of components such as fine particulate matter (PM), organic compounds such as polycyclic aromatic hydrocarbons (PAHs), gases and heavy metals. The main route of exposure to air pollution is through inhalation. PM can be divided into particles of 10 microns (PM 10) and 2.5 microns or smaller (PM 2.5), which are even more harmful to health. PM 2.5 can cross the lung barrier and enter the blood system. Chronic exposure to PM contributes to the risk of developing cardiovascular, respiratory diseases and lung cancer.

**Objective:** To evaluate the presence of metals and PAHs in urban dust samples and their impact on people's health through the evaluation of the response of immune cells and determination of DNA damage in people residing in an area neighboring the park. industrial of the city of Cartagena, Colombia.

**Methodology:** The sampling location was located near the industrial zone. 12 urban dust samples were collected. Environmental contaminants were quantified by atomic absorption spectrometry for metals and GC-MS analysis for PAHs. Biomonitoring was carried out in a cohort of thirty adult inhabitants of the region who freely agreed to participate. The contaminants were quantified and an automated count of immune cells was performed in peripheral blood to determine the leukocyte formula as a biomarker of immunotoxicity; Genotoxicity was determined with comet assay and micronucleus identification. Data were expressed as means  $\pm$  SEM. ANOVA was performed for statistical analysis. GraphPad Prism was used,  $p < 0.05$  was considered statistically significant.

**Results:** The data obtained provided evidence of the presence of substances toxic to human health in variable concentrations. Biomonitoring showed different responses to evaluated contaminants.

**Conclusion:** Biomonitoring contributes to the understanding of the mechanisms underlying adverse health impacts due to exposure to environmental contaminants.

**Keywords:** Air pollution, fine particulate matter, genotoxicity, immunotoxicity.

### **3.07 Beyond Microplastics: Analytics, Environmental Fate and Impacts of (Water-Soluble) Polymers and Biodegradable Polymers**

#### **3.07.T-01 Application of standardized methods to evaluate the environmental safety of polyvinyl alcohol disposed of down the drain**

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PVOH 18-88 is a water soluble form of PVOH. It was used as a case study to evaluate the environmental fate, ecotoxicity, and overall safety profile of polyvinyl alcohol homopolymers used in detergent films. An OECD 303A Wastewater Treatment Plant Simulation Study was conducted with dissolved organic carbon (DOC) as the analytical endpoint. During the plateau phase high levels of removal due to biodegradation were observed (avg  $97.4 \pm 7.1$ , range 88-116%). The OECD 303A simulation study quantitatively verified that freshwater is the dominant receiving compartment for PVOH 18-88 post treatment. River water screening respirometry was performed to confirm the ability to surface water microbes to degrade PVOH. A suite of standard acute algae, invertebrate, and fish embryo (FET) ecotoxicity studies were performed. Due to the potential for the chorion to impact PVOH 18-88 bioavailability, both chorionated and dechorionated FET tests were conducted.  $L/EC_{50} > 1000$  mg/L for FET (chorionated and dechorionated), invertebrate, and algae were observed. The REACH framework was used to evaluate EU environmental risk. EUSES was used to predict local and regional exposure concentrations and compared to the predicted no effect concentration (1 mg/L). The predicted exposure concentrations were  $> 2$  OM below the effects concentration, indicating negligible risk from PVOH homopolymer used in detergent films. This talk will also include an update our efforts to conduct additional simulation tests (OECD 314B and OECD 309) using tritiated PVOH 18-88.

#### **3.07.T-02 Biodegradation of Water-Soluble Polymers by Wastewater Microbiomes: Adapting Laboratory Testing Protocols**

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Biodegradability is an important functionality of water-soluble polymers (WSPs) that enter environmental systems at the end of their lifecycle. To enable the development and regulation of biodegradable WSPs, there is a need for testing methods that are scientifically valid and economically practicable. In our research, we have employed respirometric laboratory tests, based on the OECD 301 testing guideline, to investigate the biodegradation of a set of water-soluble polymers – with a focus on the poly(amino acids) poly(lysine) and poly(aspartic acid), and further including poly(ethylene glycol) and poly(vinyl alcohol). As the use of WSPs in many applications (e.g., home and personal care) results in the release of WSPs into wastewater systems, we conducted our study with microbial inocula derived from the aeration tanks of two municipal wastewater treatment plants.

Given that the OECD 301 guideline, and the majority of commonly applied biodegradation testing methods, were developed for small molecules, our main goal was to examine the effects of protocol variations on the resulting biodegradation dynamics for WSPs. Our tests revealed that avoiding aeration of the microbial inocula before introducing WSPs, subjecting WSPs to incubation with extracellular extracts from untreated wastewater prior to biodegradation testing, and reducing the concentration of WSPs all can have a positive impact on WSP biodegradation rates. While individual protocol variations resulted in modest effects, we observed a remarkable effect when combining the afore-mentioned protocol variations. Importantly, the tested protocol variations had no substantial effect on the biodegradation dynamics of low-molecular-weight reference substrates tested in parallel, suggesting that the effects are indeed specific for the WSPs. Complementary to respirometric tests, we



applied enzyme activity assays and conducted microbial community analyses to further investigate effects of protocol variations on enzymes and microbiomes used for testing.

This contribution sheds light on the relevance, strengths, and limitations of respirometric laboratory methods for biodegradation testing of water-soluble, reveals promising biodegradation dynamics of poly(amino acids), and provides an initial step in the process of adapting biodegradation testing protocols from small molecules to polymers.

### **3.07.T-03 A Systematic Approach for a Holistic Ecotoxicological Assessment Strategy of Polymers from the Laboratory to Field Scale**

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To enhance crop growing conditions and a secure yield, commercial agricultural practice resorts to e.g. seed coatings or mulch films. Those products often contain polymers favoured by their physico-chemical properties. However, the substance group is not regulated except for intentionally added microplastics, which were recently banned from the European market. Evaluation concepts for polymers, e.g. PLC-Concept, do not include ecotoxicity as indicator for environmental concern. As a result, the environmental behaviour of polymers remains unknown. Our main aim of the study is therefore to develop a systematic ecotoxicological hazard assessment including terrestrial and aquatic toxicity screening for (bio-)polymers.

To determine suitable test methods, model organisms and test systems were chosen based on REACH and Plant Protection regulation (EC 1907/2006, EC 1107/2009) considered as regulatory required standard tests contrasted to potentially more sensitive alternative tests.

For the terrestrial compartment the regulatory required tests OECD 216 (Nitrogen transformation), OECD 232 and OECD 222 (Collembola and earthworm reproduction) are compared to ISO 15685 (Potential nitrification), ISO 20130 (Extracellular enzymatic activities), molecularbiological testing (ARISA and qPCR with targets: amoA, amoB), as well as ISO 17512-1/-2 behaviour studies (Collembola and earthworm avoidance). For the aquatic compartment OECD 201 (Alga growth inhibition) and OECD 202 (Daphnia acute immobilisation) tests will be miniaturised to enhance substance throughput efficiency.

The tests will be performed with different types of polymers, i.e. biopolymers (cellulose fibres, chitosan, alginate, xanthan), synthetically modified polymers (starch, carboxymethylcellulose, hard/ charcoal), bioplastics (polylactic acid) and microplastics (polyethylene, polycaprolactam).

First results are available for modified polymers and biopolymers. A differentiation of test suitability for certain polymeric groups (biopolymers, modified polymers) is not possible yet. Terrestrial test methods indicated highest sensitivity for microbial function as well as earthworm behaviour testing. Comparing aquatic ecotoxicity tests, alga growth is the most suitable indicator for aquatic ecotoxicity as daphnia immobility was no sensitive endpoint. For final test method sensitivity comparison, the data set will be completed and presented at the conference.

### **3.07.T-04 Analytical Characterisation of Polymers for EU Regulatory Purposes**

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In 2020, the European Commission started developing a proposal for polymer registration based on Article 138(2) of the REACH Regulation. The proposal defines certain physico-chemical parameters and information on structure and composition that need to be assessed by the registrants in detail to determine if a polymer requires registration. To achieve a meaningful implementation of the potential Regulation of polymers, the identity of these complex substances needs to be established in a way that comparability of the analytical results obtained by the manufacturers and importers of the polymers is ensured. It needs for instance to be ensured that companies dealing with the exact same polymer do not end up with different conclusions on its identity. Analytical characterisation of certain polymers is anticipated to be challenging due to their chemical complexity.

Comparable analytical characterisation of the polymers to be registered is a prerequisite for their proper hazard assessment and the assessment of closely related analogues. It should be stressed that information on chemical composition, structure and physico-chemical parameters are also important features that can influence the environmental fate, bioavailability and toxicity of polymer. Therefore, correct identification and characterisation of polymers will allow to select the best representative(s) for testing under the future regulatory scheme.

Many analytical techniques exist to characterise polymers. However, if different techniques are used the comparability of the obtained results and consequently the identification of polymers might be difficult. Even if the same technique is used, certain

standardisations of the methods are needed to minimise differences in the outcomes. Finally, for such complex substances as polymers the manufacturing process description adds a significant part to the identification and characterisation.

ECHA intends to gather statistical data on the registered No Longer Polymers. The aim of the work is to assess the relation between the reported compositions and the type of the analytical techniques submitted in the dossiers, and to measure the concerns that companies rely on different analytical methods, which may be a source for inconsistencies. The work will additionally provide further insight on the strategy considered so far for characterising the low molecular weight polymerised constituents. Part of the results of this work will be shared at the conference.

### **3.07.P Beyond Microplastics: Analytics, Environmental Fate and Impacts of (Water-Soluble) Polymers and Biodegradable Polymers**

#### **3.07.P-Th280 Bioavailability of Polymers: Factors Governing Bioaccumulation and Toxicity**

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Polymers, integral components of numerous modern materials, lack the same comprehensive environmental safety assessments afforded to non-polymeric substances. Despite their widespread use, limited knowledge exists regarding their behavior in the environment, prompting the need for further exploration of properties such as bioavailability and bioaccumulation, pivotal in determining toxicity. Presently, the European Commission is actively developing a proposal to instigate the registration of polymers under the European Union's REACH chemicals legislation, reflecting a contemporary effort to enhance understanding and regulation of these versatile materials. Some sources suggest the concept of "polymers of low concern", categorized based on a set of structural and physico-chemical criteria, that would receive exemptions from notification requirements. Suggested criteria include parameters such as molecular weight, electrical charge, reactive functional groups, partition coefficients or water solubility. Are all these criteria equally relevant for predicting bioaccumulation of a polymer, or is it possible to identify a small number of key parameters? Therefore, to support this effort to develop a risk assessment methodology for polymers, the factors affecting bioaccumulation of polymers will be investigated via an initial literature search. Results to these two questions would be presented: 1) What are the influential parameters for the hazard assessment of polymers and 2) What is the correlation of the identified key parameters to bioaccumulation.

#### **3.07.P-Th281 Overcoming Analytical Challenges in Size Exclusion Chromatography for Accurate Characterization of Molecular Weight Distribution of Polymers**

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Polymers, composed of repeating monomer units, exhibit distributions in many properties, with molecular weight being the most prominent. Accurate determination of the molecular weight distribution (MWD) and oligomer content (percentage below 1000 g/mol and 500 g/mol) is essential in the regulatory framework of polymer risk assessment. Within any regulatory framework for polymers standardized, rapid, universally applicable, and validated methods for MWD determination are required. Size exclusion chromatography (SEC) or gel permeation chromatography (GPC) is the state-of-the-art technique for MWD characterization. However, SEC poses several analytical challenges in accurate determination of MWD and oligomer content.

Limitations of SEC arise for example from insolubility, crosslinking, and ultra-high molecular weight. Additionally, the interference with signals from side components such as solvents cause problems. SEC is a relative technique and thus requires suitable calibration standards, which are not always available. This limits comparability of results. For MWD determination and especially for oligomer content determination, alternatives to SEC are practically non-existent.

This presentation highlights the need for innovative methods and strategies, as well as the development of comprehensive guidelines, to facilitate the registration of polymers effectively and efficiently. Several approaches have been proposed to handle the challenges, including the use of new sample preparation techniques, data interpretation and the development of standardized methods and calibration techniques.

In summary, SEC is a well-established technique for MWD determination, but several challenges must be addressed to ensure its general applicability in a regulatory framework. Addressing the challenges associated with SEC is important for successful implementation of the upcoming REACH revision and the development of an efficient and effective polymer notification and registration process.

#### **3.07.P-Th282 A PROPOSAL FOR A THREE-TIERED APPROACH FOR TARGETED INFORMATION REQUIREMENTS FOR POLYMERS**

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Polymers are a diverse set of chemistries, part of our daily life and provide a multitude of technical functionalities. Usually, polymers cover a broad spectrum of molecules with medium to very high molecular size such as celluloses, waxes and resins. By their nature, polymers are different to small and discrete molecules and conventional risk assessment approaches are not necessarily equally suitable for polymers.

Therefore, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Polymers Task Force has developed a conceptual three-tiered proposal to generate data to assess individual and groups of polymers requiring registration (PRR). A prerequisite is the grouping of polymers according to three criteria: chemistry, physico-chemical properties and hazard similarity. The proposed approach considers the limited bioavailability of polymers as a prominent difference to many small molecules and takes this into account as a central element, in particular for human health. Among others, methods for assessing a potential for systemic bioavailability are integral to Tier 1. Within Tier 1, basic data will be generated on a single polymer or polymer group making use of non-vertebrate testing methodologies. Decisions for further studies at higher tiers are based on considerations of properties and effects, combined with systemic bioavailability, and use and exposure considerations. For many PRRs, Tier 1 data on hazard, use and exposure will likely be sufficient for achieving the protection goals under chemical legislation such as REACH. Vertebrate animal studies and higher-tier fate testing in Tiers 2 and 3 can be focussed on testing needs according to the combined factors above.

The ECETOC Polymers Task Force is of the opinion that the principle of a tiered approach with no vertebrate animal testing at Tier 1, followed by justified, limited and targeted vertebrate testing in higher tiers considering systemic bioavailability estimates, use and exposure considerations and material properties first, provides a basis for modern and considerate data generation for the next decade to ensure the selection of polymers for safe production and use. The Task Force welcomes comments and encourages scientific discussion on the proposal.

### **3.07.P-Th283 Biodegradation of Water-Soluble Polymers – Reviewing In-Silico Methods**

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In light of the increasing prevalence of polymers across diverse industries, evaluating their environmental impact becomes imperative, particularly within the framework of the European Green Deal and the Chemicals Strategy for Sustainability. Historically, there was no mandated scrutiny of the environmental fate of polymers, as they were exempt from REACH (EC 1107/2009). However, this exemption is anticipated to undergo changes in the coming years. A crucial aspect of this evaluation is the assessment of degradability and persistence of functional polymers such as for applications in cosmetic formulations.

Degradation processes are fundamentally describable through chemical formulations and can be mathematically modeled. These models can then be implemented in computer programs to calculate degradation pathways for any substance based on its chemical profile. In instances where degradation processes lack an analytically closed-form, statistical procedures, such as Quantitative Structure-Activity Relationship (QSAR) and Quantitative Structure-Property Relationship (QSPR) models, can be employed to establish relationships between the structure of a polymer and its degradation characteristics.

This poster presents the current state of in silico methods for assessing polymer degradation, shedding light on their limitations in handling polymers and in accurately calculating biodegradation. The shortcomings are examined, emphasizing the need for improvement in these methodologies. Potential avenues for enhancement are outlined, underscoring the importance of refining in silico methods to ensure a comprehensive understanding of the environmental fate of polymers, especially those used in water-soluble cosmetic formulation applications.

### **3.07.P-Th284 A Novel Approach for Determining Oligomer Content in Partially Insoluble Polymers Using Size Exclusion Chromatography and OECD 118/119**

*Timo Florian Beskers, Bastiaan Staal and Bianca Czarny, BASF SE, Germany*

Size exclusion chromatography (SEC) is a widely used method for determining the molecular weight distribution (MWD) of polymers and polymeric products. However, the analysis of high-molecular weight or cross-linked samples using SEC presents challenges, particularly in accurately determining the oligomer content. This content of lower molecular weight fractions below 1000 g/mol and 500 g/mol is vital for regulatory purposes. The method for SEC and oligomer content determination is outlined in OECD guidelines 118 and 119, as well as similar DIN and ISO norms.

The upper limit of SEC separation is determined by the pore sizes in the column material. Larger molecules or species, such as ultra-high molecular weight or cross-linked samples (gel), do not dissolve completely or only swell, impeding their passage through the SEC column. Consequently, only the soluble fraction is analyzed, resulting in underestimated mean values of the MWD (Mn, Mw), and an overestimation of the oligomer content.

Determining the proportion of the analyzed sample from the total sample proves challenging, with weighing the filter often yielding inaccurate results. It is recommended in OECD 119 to calibrate the detector response to determine the amount of the analyzed sample. However, this approach requires either known dn/dc values or a completely soluble, SEC-compatible reference substance with identical chemical composition to the sample. Both is usually not available.

In summary, the analysis of ultra-high-molecular weight or cross-linked samples - such as dispersions used in paint for example - by SEC poses difficulties, hindering the accurate determination of the oligomer content. This presents a challenge

for proper classification in the risk assessment process of polymers, as an alternative, equivalent method for determining oligomer content does not currently exist.

Here, we present a novel approach for determining the detector response and, consequently, dn/dc for partially insoluble polymers such as cross-linked or high molecular weight polymers. This new method uses ultrasound to break down the crosslinking and makes the sample fully soluble, thus enabling the precise determination of the oligomer content (amounts <1000 g/mol and <500 g/mol) with only two simple SEC measurements following OECD 118/119. Importantly, this approach can be applied even when the product is a mixture or dispersion of unknown concentration, which is often the case for real-life polymeric products.

### **3.07.P-Th285 Assessing the Applicability of Eco-Toxicological OECD Guidelines 202 and 487 for Testing of Polymers**

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In line with the recent developments for the transitions into circular and sustainable economy, the Chemical Strategy for Sustainability aims at the extension of registration requirements for chemicals. A major consideration concerns the planned end of the exemption of polymers from registration in REACH.

The generation of robust, meaningful and consistent data as well as from analytical and from toxicological testing is paramount for this purpose. OECD guidelines exist for such purpose, but were developed for small, well-defined molecules and their applicability to much larger polymeric species is questionable. The main problem is the high molar mass that is intrinsic to many polymers. Additionally, polymers are characterized by a distribution of molecular weights, potentially also a distribution of composition.

Such nature differentiates them from the small molecules, resulting in different behaviour in test media (e.g., solubility, stability, reactivity), all whilst the sample preparation procedure becomes even more important. In general, several cases can be identified:

- polymers are soluble in test media, but may still interfere with assays e.g. due to light scattering
- polymers are insoluble in media, but forming a stable dispersion of particles, resulting in a constant exposure during ecotoxicological tests
- insoluble polymers, unstable in test media
- and polymers reacting with water and/or test media.

Here we demonstrate results obtained for a set of polymers that represent each of the above categories with different properties (molecular weights, chemistry) when one applies the test media, concentrations and procedures prescribed in OECD guidelines for aquatic toxicity (OECD 202) and genotoxicity (OECD 487). We applied different sample preparation procedures and tested the colloidal stability and homogeneity to get insight into polymer behaviour during actual testing. These results are compared to the observations during toxicity testing and evaluation of water solubility of polymers according to the OECD Guideline 120.

### **3.07.P-Th286 Influence of Gel Permeation Chromatography Set-up on Results**

*Sebastian Schmiedt and Paul Bendig, Eurofins Agroscience Services EAG Laboratories GmbH, Germany*

For registration of Polymers under REACH characterization of polymers with gel permeation chromatography (GPC) will be a prerequisite. The technique appears well established to determine molecular weight distributions of polymers itself. However, complexity increases when looking at different chromatographic conditions and low molecular weight fractions.

It has been reported that the separation of analytes and the results obtained using gel permeation chromatography is not solely depending on the molecular weight, but also other attributes. Hence, results are highly dependent on the combination of column material, elution solvent and calibration substances and naturally on the polymer itself.

The influence on results of selected column-solvent system combinations is presented and applied to different example polymers. Challenges and opportunities in the low molecular weight fraction will be investigated and discussed. The low molecular weight fraction may comprise monomeric and oligomeric constituents/impurities as well as intentionally added chemicals. Combination of different detectors could help enhance comprehensibility of the elugram in the low molecular weight fraction.

### **3.07.P-Th287 Challenges and Recommendations for Addressing Side Components in SEC Chromatograms of Polymers for Regulatory Purposes**

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Size Exclusion Chromatography (SEC) is a widely used technique for analyzing the molecular weight distribution (MWD) and

oligomer content of polymers. Accurate determination of oligomer content is important within regulatory risk assessment frameworks for polymers. It's part of the basic data requirement for polymer substance registration in many countries, including the anticipated REACH revision in the EU. SEC separates molecules based on their size in solution, specifically their hydrodynamic volume, making it an entropic process that is not specific to any particular chemical characteristic.

However, the presence of side components in the sample poses a significant challenge in SEC analysis. SEC measurement conditions must be optimized for the polymer species, striking a balance in polarity between the sample, solvent, and stationary phase to prevent enthalpic interactions that could impede the SEC separation mechanism. Side components can be of different chemical natures and potentially different polarities. If these side components have similar polarity, they elute within the calibrated SEC range, overlapping with the polymer signals and hindering accurate determination of oligomer content and molecular weight distribution. However, if side components have different polarity, they either elute after the SEC chromatogram and the void peak (similar to other forms of liquid chromatography) or may not be detected at all if they are insoluble in the solvent for example. Consequently, oligomer content determined using SEC cannot serve as a comprehensive parameter for all low molecular weight species present. Neither does SEC always measure the oligomer content of the polymer alone, as it lacks specificity. Analyzing real-life polymer products, where the contained polymer is often not available in pure form, presents challenges due to overlapping peaks. In some cases, polymer purification or careful selection of the LC method parameters can induce interactions with side components, causing their peaks to fall outside the SEC separation range. Alternatively, if the concentration of the side component is known and can be measured as a reference, peak subtraction can be employed as a viable solution.

In this presentation, we will discuss the challenges, provide examples, and offer recommendations for effectively addressing side components in SEC analysis.

### **3.07.P-Th288 Polyvinylpyrrolidone in aquatic organisms: Development of an aqueous GPC method for water-soluble polymer analysis**

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Water-soluble polymers (WSPs) are additives that are widespread in both domestic and industrial products due to their plethora of uses and characteristics in formulation. These include personal-care products (PCPs) as well as wastewater treatment flocculants, soil conditioning additives and as an ingredient in aquaculture products. These polymers are widely used and therefore predicted to enter the environment, with polymers such as polyethylene oxides (PEOs) quantified in freshwater. However, there is a limited understanding of their environmental effects, partially due to the lack of regulation surrounding their use but also due to their complex and vast range of chemical structures and behaviour in environmental matrices. Polyvinylpyrrolidone (PVP) represents a particular concern due to its lack of biodegradation, which implies it may have the ability to persist in the environment and has already been shown to have sub-lethal toxic effects at environmentally relevant concentrations on multiple species during lab-based studies. Despite the increased interest in PVP, there is still a lack of quantitative information to inform on ecotoxicological persistence, due to the lack of analytical methods to detect PVP. This is relevant for environmental protection and regulation, but its prevalence within aquaculture practises to prevent disease outbreak raises direct concern for food security and human ingestion of polymers. Multiple methods have begun to be developed for WSP analysis, however this is still with a focus on lower-molecular weight polymers. Polymer specific methods are only very recently being explored as an alternative, combining traditional polymer analysis methods such as gel-permeation chromatography (GPC) with ecotoxicology. In this study, we explore the use of GPC as a method to detect and quantify PVP in exposed aquatic organisms to determine whether bioaccumulation of this chemical is possible in aquatic food webs. This ongoing work aims to investigate both the analytical capabilities of GPC in environmental analysis but also explain the sub-lethal toxicity of PVP to aquatic species.

### **3.07.P-Th289 A comprehensive study of polymer – lipid membrane interactions with in-chemico NAMs.**

*Coralie Sophie Schneider<sup>1</sup>, Wendel Wohlleben, Dr.<sup>2</sup> and Lan Ma-Hock<sup>2</sup>, (1)BASF, United States, (2)BASF SE, Germany*  
To minimize the need for in-vivo testing with animals, new approach methods (NAM) and integrated assessment strategies are needed for potential hazard and/or bioaccumulation. One such strategy could include the testing for interactions between polymers and model bio membranes (ECHA, Key Areas of Regulatory Challenge, November 2023). Our test set included both solid polymers (i.e. nano- and microplastics) and soluble polymers with many different chemical functionalities.

We present a comprehensive study of polymer – lipid membrane interactions and quantification thereof consisting of three different in-chemico NAMs: Firstly, with the quartz-crystal microbalance with dissipation monitoring (QCM-D), where we have prepared a supported lipid bilayer (SLB) at the solid/water interface. Secondly, with interfacial rheology, where a lipid layer is prepared at the water/air interface of a drop. And lastly, with analytical ultracentrifugation we test the interaction between unilamellar lipid vesicles and polymers by detecting the disruption, insertion, or bridging of vesicles. Additionally, electron spin resonance with CPH spin trap (EPR) experiments were performed to detect radical generation as a potential initiating event of cellular toxicity (Lehman et al, Environ. Sci.: Nano, 2016, 3, 55-56).

Since the four methods test for different interactions via different mechanisms or at different interfaces, a comprehensive data set was generated. To assess the relevance of the different descriptors for prediction of toxicity, data sets of selected polymers were compared to and correlated with existing in-vivo inhalation data.

### **3.07.P-Th290 Polyethylene Terephthalate (PET) Biodegradation Using Isothermal Titration Calorimetry: A Path to Circular Economy**

*Noelia Fernandez Merayo<sup>1</sup> and Thomas Maskow<sup>2</sup>, (1)Environmental Microbiology, University of Leipzig, Germany, (2)Environmental Microbiology, Helmholtz Centre for Environmental Research (UFZ), Germany*

The increasing plastic production has been a concern for the last decades, reaching a production of 390.7 million metric tons in 2021, excluding polymers that are not used in the conversion of plastic parts and products (i.e. for paints, cosmetics, textiles, adhesives, etc.). As a consequence, the current plastic waste management system has been put on the spotlight, since according to the OECD only 9% of the plastic waste is being recycled. Subsequently a large fraction of the plastic enters the environment, which afterwards will most likely be fragmented and turned into microplastics (<5 mm) and nanoplastics (NP, <100 nm). In addition, NP are of higher concern as they can permeate into animal and human tissues.

Polyethylene terephthalate (PET) is of particular interest, since it is mainly used for packaging and disposable items and therefore often ends up in the environment instead of being recycled. It has been reported that PET-NP can be degraded by different microorganisms or enzymes (e.g. *Ideonella sakaiensis*, TtCut2 from *Thermobifida fusca*) to obtain intermediate oligomers or the original monomers, i.e. terephthalic acid and ethylene glycol. The technical exploitation of these potentials would open up options for efficient recycling and approaching to a circular economy. This requires information about kinetic and thermodynamic parameters of the depolymerization, hard to obtain with conventional analytical techniques.

Using isothermal titration calorimetry, it has been possible to monitor and characterize the biodegradation process. A model was developed for the degradation of PET nanoparticles size 180 nm, using a plant-metagenome-derived cutinase, the LCC<sup>ICCG</sup>. This provides a new method, along with insight into the kinetic cleavage of ester bonds, opening the door to implementing this at a bigger scale and a possibility of expanding this models to different hydrolysable polymers.

### **3.07.P-Th291 Polymer risk assessment: Consideration from the ECETOC Polymer Task Force**

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Polymers represent a large and broad aspect of the chemical space and provide a multitude of technical functionalities. An ECETOC Task Force has summarized the state-of-the-art polymer regulation and testing. Importantly, the Task Force has proposed the ECETOC Conceptual Framework for Polymer Risk Assessment (CF4Polymers) that includes eight steps for aspects to consider in risk assessment and intelligent testing of polymers. Case studies confirmed that due to the wide range of polymer types and properties, no 'one size fits all' set of rules is applicable to this diverse set of materials, leaving regulators and industry to apply rules on a case-by-case basis. The scope of the risk assessment of polymer products may vary depending on a combination of deciding factors, among which the assessor considers the type of polymer chemistry, the application during use and relevant routes of exposures. Depending on scope, the focus may be on the polymeric substance should the polymeric substance have reactive functional group suggesting specific hazards. Alternatively, emphasis may be on IAS (intentionally added substances) and NIAS (non-intentionally added substances). Indeed, many polymer types are complex mixtures where IAS and NIAS contribute to, and in some cases are responsible for, the expressed hazard properties. Currently in the EU, product regulations such food contact materials and cosmetics focus on the product as placed on the market and prioritize constituents of polymeric products relevant to potential exposure. The objective of expanding the registration of polymers, for example in the REACH regulation, raises questions on the type of information required to support the purpose of the safety assessment of polymers requiring registration (PRR), as produced. This results in a shift of paradigm from a focus on a targeted scoping of assessment on polymer products aligned to the end use applications, to standard information requirement for PRR. This challenging landscape led the ECETOC Task Force to develop a tiered approach to support the assessment of PRR, considering elements of bioavailability to biota and the objective of minimizing vertebrate testing. Challenges in informing quantitative risk assessment will be discussed, acknowledging the lack of agreement among stakeholders on the domain of applicability of test methods, as well on the suitability of testing information to support the safety assessment of polymers.

### **3.07.P-Th292 Environmental Persistence Assessment for Synthetically Modified Biopolymers**

*Harald Streicher<sup>1</sup>, Diego Robles<sup>1</sup> and Alistair Boxall<sup>2</sup>, (1)Beiersdorf, Germany, (2)University of York, United Kingdom*

Synthetic polymers are used in a very broad range of products and applications. In the area of cosmetic products, they enable important functionalities of e.g., rheology modifying and film forming. However, established materials are often known to be persistent in the environment. In contrast, natural biopolymers e.g. Cellulose, Starch, Guar are biodegradable but both their performance as well as their variety of resulting sensorial profiles are limited and not up to par to conventional technologies.

Synthetically modified biopolymers are an interesting and promising alternative for cosmetic product formulations as they combine several aspects: Their natural polymer backbone is biodegradable and their functional hydroxyl groups allow a wide range of synthetic modifications and functionalities. The current screening tests to rule out persistency (e.g., OECD 301 and OECD 302) were not developed for polymers. As polymers are exempt from registration e.g. via REACH in the EU, data gaps are frequent.

To ensure essential biodegradability a weight of evidence persistency assessment is proposed which takes into account OECD screening results, structural analysis of the respective synthetically modified biopolymer and higher tier testing results.

Biodegradation data of different synthetically modified celluloses with different functional groups and molecular weights has been generated to compare OECD screening results with higher tier testing data.

### **3.07.P-Th293 Safer by design approach to support innovation : a practical case study with microplastics**

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Solvay aims to develop different activities to assess the potential impact of the substances during their manufacturing, their use or their degradation/release. A safer-by-design approach allows the selection of the best candidates from application performance tests, (eco-)toxicological assays and biodegradability results, and is employed during the development of a new range of safe and sustainable products by our research and innovation function.

One of the safer by design approaches for which we gather information at an early stage of development is biodegradability evaluation. According to the application, the substances (coating of seeds, additives in shampoo...) can be released in the environment or in wastewater treatment plants. In this context, the research lab aims to investigate the biodegradability of the product, by following the degradation *via* respirometric analyses from micro-organisms.

Among the substances which have gone through this process during the past years, some microplastics case studies are presented here. Polymers are used in a wide range of applications such as packaging and coating, consumer goods, medical equipment or agriculture. Polymers with a low solubility below 2g.L-1 can be suspected to be microplastics according to the new incoming regulation in Europe. To answer to the needs of their registration and classification several factors such as their ready and/ultimate biodegradability can be highlighted in conventional OECD tests to ensure their environmental safety and to confirm that they can be considered or not as microplastics. Among those tests, our internal facilities allow Solvay to screen both ready biodegradability traits with or without BIMs (bioavailability improvement methods) and notably (emulsion) to highlight the potential of biodegradability of the substance. If these first tests are not conclusive, OECD & regulations propose to investigate both compartmental biodegradability and potential of persistence of these substances.

Associating to these biodegradation results, the impact of the native substance and of its biodegradation byproducts in terms of toxicity and ecotoxicity, will allow in the future to secure our safer by design approach.

### **3.07.P-Th294 Modelling Techniques for the Prediction of Polymer Properties**

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The development and use of *in silico* tools for the modelling and prediction of polymer properties has evolved rapidly over the past decade, mainly based on evolution of machine-learning (ML) techniques. These techniques allow fast and accurate prediction of factors of interest, which has already been well documented. The use of predictive models for environmental fate in the regulatory context is already well established, with the use of BIOWIN, EPISUITE and OPERA already commonly used for the assessment of environmental fate and pathways. So far, however, these tools are only available for the evaluation of small molecules, as polymer structure is frequently too complex to accurately use for ML. Techniques used for small molecules – such as SMILES or InChI codes - cannot convey the information on polydispersity, branching, crystallinity or macroscopic structure which polymer modelling requires for accurate understanding. The lack of comprehensive datasets containing this information has additionally hindered development of models for polymers. As the environmental fate of polymers is receiving growing attention, it is increasingly important and relevant to develop tools which apply to this class.

A number of new methods are now being proposed which show promise in offering a description which can be used by ML. BigSMILES is one of the most popular, as its logic and notation extend from the already widely used SMILES. There is however still much development still needed on an overarching method to describe polydispersity, for example. Here, new applications of ML will be presented which can be used to predict environmental fate of polymer structures. More importantly, a framework will be given on what is still needed in order to develop ML for polymer environmental fate applications into tools for regulatory purposes.

By developing new methods to accurately describe polymer structure *in silico*, it will open the door to development of new ML based tools for prediction of environmental fate for complex structures. Such tools will greatly increase the amount of data available for fate of new structures in different environments, and critically, will offer fast, low-cost tools which aid understanding before higher cost lab tests.

### **3.07.P-Th295 Applying a High-Throughput Biodegradation Test to Assess Factors Affecting the Biodegradability of Polymers**

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Polymers are found in a wide variety of everyday products from pharmaceuticals to detergents, this results in a high environmental exposure upon their disposal. Despite this, little is known about the fate of these polymers in the environment and there is only a limited range of tools available to assess their biodegradability. To address these two problems, we have developed a new high-throughput biodegradation test for glucose-based biopolymers. This method detects and quantifies glucose as the analytical endpoint of the polymer's biodegradation and can be conducted in a 96-well plate format. It exposes the polymer to an enzyme-mixture specific to its backbone, and under ideal conditions for the enzymes, to see how much degradation can be achieved in 48 hours. To optimise this for eventual use on complex modified polymers, the test was applied first to unmodified polymer backbones, in particular Pullulan and Dextran. The aim was to detect approximately 100% degradation in these unmodified polymers to be confident that the method was reliable enough to test modified counterparts. Thus far, this has been achieved in Pullulan but not Dextran which is hypothesised to reach ~100% with adjustments to the choice of secondary enzymes. Once optimised, this test could then be utilised to study the effects of different characteristics on the biodegradability of polymers. A set of modified Pullulan and Dextran samples with differences in degree of substitution and molecular weight have been selected for testing, to investigate the effects these characteristics have on their biodegradation. It is further hypothesised that degree of substitution will have a greater effect with greater levels of substitution lowering biodegradability. Once completed, this method could be a simple, high-throughput tool for providing an insight into biodegradability in novel polymers, before committing to more expensive and laborious biodegradation testing. Applying this test could also advance knowledge of factors affecting biodegradability of these polymers to help better understand their fate in the environment.

### **3.07.P-Th296 Investigating Biodegradation of Polymers: A Bibliometric Analysis of Research Trends and Proposed Informative Framework for Water-Soluble Polymers**

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There is a growing global concern around chemicals in consumer products, given the associated demand and the potential risks they pose to the environment and human health. Many of these chemicals originate from petrochemical feedstocks. In the pursuit of sustainable alternatives, attention is turning to polymers. While plastic polymers have extensive information, there is a research gap in bio-based polymers, which present a promising substitute for fossil-fuel-based chemicals in consumer products.

This study investigates the research and grey literature to better understand polymer characterization analysis, particularly in the context of biodegradation. The overall goal is to develop an information-based framework for making decisions about developing appropriate biodegradation tests for polymers. The bibliographic analysis involved extracting literature from SCOPUS using VOS viewer software and EndNote. This analysis narrowed down the initial 9593 publications to 157 papers, which were thoroughly reviewed to identify research trends and critical knowledge gaps.

The quantitative analysis showed a deficiency in research focus, particularly regarding biodegradation tests, polymer fate, distribution of potential polymer-degrading microorganisms, and methods for quantifying and handling degradation products. Furthermore, critical knowledge gaps were identified, emphasizing the absence of standardized testing methods for biodegradable polymers (BP), and the lack of persistence assessments, hindering accurate evaluation.

We have proposed an informative framework to understand crucial polymer characteristics and biodegradation, enabling decisions based on accurate test information, analytical endpoints, and diverse testing strategies. This proposal encompasses various stages: 1) Polymer selection; 2) Information collection; 3) Evaluation of methodology and applicability; 4) Identification of test limitations; 5) Analysis of fragments and transformed derivatives; 6) Investigation of long-term fate and test modifications. We intend to further develop and evaluate this framework, utilizing the case study of water-soluble polymers and validating it through laboratory experiments, which can be used in down-the-drain consumer products.

This research significantly contributes to the field by offering a roadmap for future investigations on biodegradable polymers. Ultimately, it addresses a crucial aspect of environmental sustainability for future generations.

### **3.07.P-Th297 Fragrance encapsulates: impact of the polymeric shell purification method on the accuracy of the OECD 301F biodegradability assessment**

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Fragrance encapsulates are widely used in consumer care applications such as fabric softeners or other liquid laundry care



products since they provide multiple benefits, from fragrance protection in the commercial product to a controlled release and improved sensorial experience for the consumers. Polymeric fragrance encapsulates are in scope of the EU regulation restricting the use of intentionally added microplastics particles and industry is actively working on innovation programs to find biodegradable alternatives. However, particular attention needs to be paid to claims that a fragrance encapsulation system is biodegradable because biodegradation test results can vary considerably depending on how a test material is prepared, which can even lead to false positive biodegradation test results. In this study we demonstrate the importance of the sample preparation phase of the process. We show how the biodegradation level can fluctuate from 0 to 91%, depending on how the test material is isolated from a given microcapsule slurry system and present a method that can be used in order to obtain trustworthy biodegradation results.

### **3.07.P-Th298 (Bio)degradation of Model Polymers with Varying Physico-Chemicals Properties & Structures**

*Guillaume Cottin<sup>1</sup>, Elsa Paiva<sup>2</sup>, Xavier Schultze<sup>2</sup>, Jérôme Lorient<sup>2</sup>, Sean Ellacott<sup>2</sup>, Alaa Bensetra<sup>3</sup> and Cyril Sweetlove<sup>1</sup>, (1)L'Oréal, France, (2)L'Oreal Research & Innovation, France, (3)Analytical chemistry, L'Oréal, France*

The life cycle of polymers most commonly found in the environment has been estimated at several decades, even hundreds of years. Alongside these polymers, which are mainly used in packaging, polymers that are soluble or dispersed in water or application solvents are commonly used in a wide range of industries. Considering this, chemical product regulations (including polymers) are evolving. The European Commission has notably adopted its sustainable strategy, which constitutes a key commitment of the Green Deal. In addition, the REACH and CLP regulations will be revised in the coming years and will include polymers. In this context, polymers contained in rinsed-off products require an appropriate assessment. Only polymers whose biodegradability in the environment can be demonstrated will ultimately be authorized.

In this context, the aim of this study is to establish structure-property relationships among different molecular structures (different backbones & end groups), their physico-chemical properties (T<sub>g</sub>, molecular weight, solubility, etc.) and biodegradation measured using biotic and abiotic tests. Three families of model polymers were selected (PVA (Polyvinylalcohols) / Polyalkylglycols (i.e. PEG) / PCL (Poly(caprolactone))).

These results show the importance of taking into account the physicochemical parameters of polymers (solubility, Mw, particle size, end groups, ...) in the context of the biodegradability assessment and generally in their environmental impacts assessment. These parameters generate additional variability on top of the existing biological variability.

For this, it's essential to ensure a good bioavailability of a polymer under the test condition (grinding, BiM) and include a positive polymer reference indicating an equivalent response of the activated sludge from one biodegradability test to another.

Understanding the (bio)degradation mechanisms of polymers is essential in order to optimize existing guidelines for polymers, particularly in view to regulatory evolutions.

### **3.07.P-Th299 Interactions of cosmetic polyacrylic-based water-soluble polymers with activated sludge: Biodegradability, adsorption, and effects**

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Water-soluble polymers (WSPs) are used in a variety of applications, including as an essential ingredient in personal care products and cosmetics. After use, they are flushed down the drain and treated in wastewater treatment plants. However, their interactions and fate in wastewater treatment plants are still unclear. In this context, the aim of this study was to investigate the adsorption potential of two polyacrylic acid (PAA)-based WSPs (homopolymer and copolymer) on activated sludge, their effects on settling and respiration, and their biodegradability.

The results showed that both the homopolymer and the copolymer were rapidly adsorbed on activated sludge after the first minute of contact. They were not desorbed in the following six hours of the experiment. The adsorption led to an increased settling behaviour (expressed as sludge volume index, SVI) in the case of the copolymer, while the SVI of the homopolymer was comparable to that of the blank. Neither PAAs had an effect on the respiration of the activated sludge. However, both PAAs showed limited biodegradability and did not even exceed 10 % in 28 days in the biodegradability tests.

The results showed that the selected PAAs had no acute significant effects on the activated sludge, but both showed a very high affinity to the activated sludge and were not biodegradable. The long-term effects on the activated sludge and the efficiency of the wastewater treatment plant should be investigated in future research. Due to the high adhesiveness of both PAAs, there is a possibility that PAAs can be transported together with activated sludge into the terrestrial environment if activated sludge is used as soil fertilizer or stabilized by composting.

### **3.07.P-Th300 Prolonged test duration for testing the biodegradability of polymers in OECD 301 improves reproducibility of test results**

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Polymers are currently receiving increasing regulatory attention in the EU. Examples are the planned inclusion of polymers into the scope of REACH and the restriction on intentionally added microplastics. The results of biodegradation tests are of great relevance for derogating polymers from restrictions. They also might become relevant when polymers have to be tested under REACH. Screening tests to evaluate biodegradability were developed for water-soluble, low molecular weight substances. Here, the applicability of such screening tests to polymers is explored. Recently published biodegradability test data (Mc Donough et al. 2023) are investigated in view of factors influencing the test outcome. In this study, different water-soluble polymers were tested by independent test institutes in the classic screening tests according to the OECD 301 series. Particular attention was paid to the test duration. The mean degree of degradation increased with the duration of the test. Above all, the variability of the results (variance) after 60 test days is significantly lower than after a test duration of 28 days. These findings are discussed in view of adapting the current screening tests to enhance their applicability for the assessment of polymers.

### **3.07.P-Th301 Biodegradability of Water-Soluble and Water-Dispersible Polymers in Respirometric Laboratory Methods**

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Water-soluble and water-dispersible polymers can be found in a large range of applications in our everyday life, from cosmetics to detergents, from paints to agricultural formulations. For polymeric materials regulations world-wide are rapidly developing with greater focus on the assessment of the degradability of polymers in application leading to environmental exposure. This has put an increasing emphasis on the need to identify, and critically evaluate testing guidelines for assessing biodegradability of for polymeric materials.

Multiple biodegradation testing methods are available. While developed for discrete substances, these may in principal be used to assess biodegradability of soluble and poorly soluble polymeric materials. These methods range from screening tests such as the OECD test guidelines (e.g., 301/310) for biodegradation, or media-specific methods such as ISO (e.g., 14852) and ASTM test guidelines (e.g., 5988). These methods have some commonalities such as reliance on non-specific analyses (i.e., O<sub>2</sub> consumption, DOC and CO<sub>2</sub>) and offer some level of flexibility regarding inoculum type and concentration and test substance dose level. Unfortunately, systematic investigations about their applicability to different types of polymers are lacking and limited publicly data are currently available. In view of the extremely dynamic regulatory atmosphere, these knowledge gaps need to be urgently filled.

In this study we present a series of experiments performed in different laboratories across the globe according to OECD, ISO and ASTM methods to study biodegradability of representative water-soluble and water-dispersible polymers. A set of synthetic polymers and modified polysaccharides have been used as case studies. Standard methods and modifications thereof to improve method deficiencies, ranging from polymer, inoculum ratio, microbial concentration and effect of temperature will be presented.

### **3.07.P-Th302 From Proteome to Behaviour: Evaluating the Ecological Impacts of Water-Soluble Polymers on *Daphnia Magna***

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Nowadays, water-soluble polymers (WSPs) are considered almost ubiquitous in the aquatic environment due to their widespread use in various industrial and consumer applications. For this reason, the presence of these polymers in the aquatic ecosystem raises concerns about their impact on the health of freshwater organisms, including keystone species such as *Daphnia magna*. This study evaluates for the first time the effects of the most used WSPs, as the polyvinyl-alcohol (PVA), polyvinylpyrrolidone (PVP), polyacrylic acid (PAA) and polyethylen glycole (PEG), on different levels of biological organization within this freshwater model organism.

An integrated approach was conducted to assess the impacts of three concentrations (0.001, 0.5 and 1 mg/L) of these WSPs at molecular, cellular, physiological, and organisms' level of *D. magna* specimens. Both acute (mortality) and chronic (heart rate and some swimming performances) effects were evaluated on the exposed organisms. In addition, the activity of monoamine oxidase (MAO) and acetylcholinesterase (AChE) were investigated to evaluate potential neurotoxic effects, while the content of glycogen (GLY) was measured to assess the potential alteration in energetic metabolism. Furthermore, a *high-throughput* methodology based on gel-free proteomics was applied on specimens exposed to the highest concentration (1 mg/L) of all the WSPs.

Our findings reveal that exposure to different WSPs induces specific responses at each biological level of *D. magna*. PEG was the only WSP inducing lethal effects at 0.5 mg/L and altered AChE activity. At the physiological level, all WSPs affected the behavioural performance and the heartbeats of the specimens, with PAA as the most impacting polymer on behavioural parameters analysed. Otherwise, proteomic analyses revealed altered protein profiles following the exposure of all the WSPs, highlighting PVA as the most effective in modulating protein expression. This study underscores the complexity of the risk assessment of these WSPs and highlights the need for a better understanding of their impact on aquatic organisms. This knowledge is crucial for the preservation of freshwater ecosystems by an informed environmental management.

### **3.07.P-Th303 Assessing the biodegradability of water-soluble polymer flocculants and their impact on microbial communities**

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Water-soluble polymers (WSPs) are chemicals ubiquitous within society. Their use in household products, such as personal-care products (PCPs), raises concerns about their entry into freshwater environments. However, their use in industry is on a much greater scale, particularly in wastewater treatment, where a large volume of WSP flocculant is used to dewater sludge before, in some cases, it is applied to agricultural land. Little is known about the chemical composition, behaviour and fate of these flocculants. Furthermore, it is not known whether they are biodegradable, either during wastewater treatment or in the environment however, their large molecular size indicates this may not be possible. To investigate this, we propose both a short and long-term biodegradation assay using biological oxygen demand (BOD) to monitor the biodegradation of multiple WSPs from microbial communities isolated from activated sludge. This includes two commonly used wastewater treatment flocculants. Using a short-term assay enables the rapid testing of readily biodegradable polymers, while a longer 28-day OECD biodegradation test continues the monitoring of more recalcitrant polymers. Alongside this, high-throughput sequencing allows the change in these microbial communities to be monitored, thus indicating the impact of WSPs on the behaviour of these communities and their ability to function. Gel-permeation chromatography (GPC) will also be used to confirm the molecular weight of the original polymer and identify any molecular weight changes throughout the study. This ongoing work aims to couple the chemical analysis of WSP biodegradation with the wider impact on microbial community composition. By identifying if wastewater flocculants are able to be biodegraded within wastewater treatment plants, we can determine if they end up in the environment and any potential concern this poses for aquatic organisms.

### **3.07.P-Th304 Fate of water-soluble and water-dispersible polymers used in agricultural formulations**

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Modern agriculture faces the challenge of ensuring a stable food supply amid climate change and freshwater scarcity, necessitating the adoption of innovative technologies in crop production. This contribution reports on the environmental fate of biodegradable water-soluble polymers (WSPs) and water-dispersible polymers (WDPs) used in agricultural formulations. WSPs and WDPs stabilize active ingredients such as pesticides and fertilizers in agricultural formulations. The environmental fate of WSPs and WDPs remains poorly studied and understood. We explored the adsorption behavior, transport dynamics, and biodegradation dynamics of conventional and candidate biodegradable WSPs and WDPs in soil. Studying the interplay between biodegradation and adsorption is critical, as adsorption to soil particles affects biodegradation rates: on the one hand, adsorption may impede biodegradation by protecting the polymers from enzymatic and microbial breakdown in soil pore waters. On the other hand, adsorption may increase the polymer concentrating in microbially active, upper soil layers and prevents transport towards less-active deeper soil horizons. Utilizing a combination of solution-depletion measurements, surface adsorption techniques, and column transport experiments, complemented by respirometric testing in soil incubations, we characterized the influence of polymer properties on their fate in soil. Our findings demonstrate that electrostatic interactions and hydrogen bonding govern adsorption of these polymers to mineral surfaces, which in turn affects their biodegradability. For polyethylene glycols (PEGs) – with seemingly contrasting biodegradation properties reported in the literature – we found decreasing biodegradation rates with increasing molecular weight suggesting that biodegradation is controlled by decreasing cellular uptake with increasing size. The contribution highlights the need to consider WSPs and WDPs chemical structure and conformation in assessing their fate in soils. A detailed understanding of adsorption and biodegradation is key to design and use sustainable WSPs and WDPs in agricultural formulations and to mitigate environmental impact.

### **3.07.P-Th305 Can we Spectroscopically Trace Deuterium from Labeled Biodegradable (Micro)Plastics into Microbial Biomolecules?**

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Complete biodegradation of plastics which cannot be retrieved from environment is essential since nano- and microplastics can have biological, physical, and chemical effects. The plastic's final utilization must be understood to study its final fate in different environments. While mineralization into CO<sub>2</sub> has been reported several times with <sup>13</sup>C-labels for reliable quantification, Zumstein et al. additionally traced the label into fungal biomass with nanoscale secondary ion mass spectrometry. We chose cost-reduced D-labels instead and monitored their uptake from deuterated polylactic acid (dPLA) into single bacterial cells with stable isotope Raman microspectroscopy. This technique was previously used to trace stable isotopes

from other carbon substrates or D<sub>2</sub>O into microbial biomass. C-H vibrations of biomolecules like lipids and proteins are extensively red-shifted into the Raman-silent region if hydrogen is replaced by its heavier isotope. Incubation experiments of an environmental bacterium with dPLA showed undeuterated biomass spectra, dPLA particles and after three weeks, additional signatures were assigned to deuterated biomass. Compared to reference spectra of bacteria labeled with glucose-d<sub>12</sub> or D<sub>2</sub>O, it has a larger D-lipid to D-protein ratio, which suggests an adapted metabolism. The cells were deuterated to up to 22 % after incubation with dPLA, and their distribution resembled that one of labeling with 50 % deuterated glucose. The next step is to extend our approach to realistic conditions of terrestrial environments. Therefore, soil microcosm experiments will be conducted with dPLA in environmental soil, which was previously exposed to PLA to allow bacteria adaptation and shorten incubation times. Until now, the isolation of cells from soil was completed to gain Raman spectra with satisfyingly small backgrounds and high signal-to-noise ratios.

### **3.07.P-Th306 Development of a Modified Screening Method for Degradation of Polymers and Validation with <sup>14</sup>C-radiolabelled Alginate**

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The fate of polymers has been in the focus of the new restriction (Commission Regulation (EU) 2023/2055) issued by the European Commission on intentionally added microplastics to products used for specific purposes. The restriction is based on the biodegradation of polymers, which is determined with the help of OECD test guidelines (TG), for example TG 301 and TG 307. It only considers synthetic polymers. Therefore, producers strive to shift to natural polymers, which are considered degradable by default, and those synthetic polymers which show proof of fast biodegradation. The biodegradation rate and formed degradation products of a synthetic polymer are mostly unknown. During product development a fast screening is needed to obtain a projection of the polymer's biodegradation potential to decide if the polymer can be considered for the product.

In order to address the degradation of polymers a modified screening method has been developed, based on the OECD 301B guideline. As modifications the test volume was reduced, the duration of the experiment was shortened and a different measurement endpoint was chosen, which led to an adjusted experimental set-up. The chosen set-up saves time and space. Instead of the mineralisation the recovery of the total organic carbon content is measured. The modified screening method showed comparable results to the OECD 301B guideline and can be used to quickly determine if further testing for a polymer during product development is relevant.

For validation of the modified screening method <sup>14</sup>C-radiolabelled sodium alginate was used. The synthesis of the radiolabelled alginate was done by a nucleophilic substitution (S<sub>N</sub>2) of the alginate's hydroxyl groups with <sup>14</sup>C-radiolabelled 2-bromoethanol. Post-synthetic chemical modification of polymers may represent a facile access to different natural <sup>14</sup>C-radiolabelled polymers. Care must be taken to reduce the impact of the derivatisation on the physico-chemical properties and the biodegradation of the polymer. However, if performed successfully the resulting <sup>14</sup>C-radiolabelled polymers can significantly improve the determination of the environmental fate of polymers. Details of the synthesis procedure and characterisation will be presented as well as the results of the validation experiments.

### **3.07.P-Th307 Downscaling of syntheses and preparation of a variety of <sup>14</sup>C-labelled polymers and oligomers**

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The use of <sup>14</sup>C-labelled substances in environmental studies is highly advantageous, because it enables the widely matrix independent detection of the parent substance and to follow resulting transformation products. While it is a well-established procedure for organic low molar mass compounds, it has rarely been applied for the investigation of environmental fate of polymers and oligomers, yet. This is mainly due to two principal challenges, first the necessary downscaling to the required small scale and second the selection and availability of the required <sup>14</sup>C-labelled monomers as the radioactivity might introduce undesired reactions of the often highly reactive monomers before the polymer synthesis starts.

Though there is no "off the rack" solution that suits for all polymers, it is possible to develop downscaling procedures for a variety of polymerisation processes and to circumvent problems related to the lack of availability of certain <sup>14</sup>C-labelled monomers. Examples for successfully synthesized substances will be presented, such as <sup>14</sup>C-labelled variants of cationic poly(acrylamide) copolymers, crosslinked calcium polystyrene sulphonate, propoxylated oligomers from a phenol-formaldehyde condensation and poly(ethylene). In each case the benchmarks for the <sup>14</sup>C-labelled polymers produced were the commercial non-labelled analogues to prove successful synthesis.

For the acrylamide copolymer, radical initiation induced by radioactivity had to be considered being a potential problem for the synthesis. The synthesis of crosslinked calcium polystyrene sulphonate required fine-tuning of dispersion polymerisation and

sulphonation processes. Similarly, the synthesis of the propoxylated oligomers had to be adapted to appropriate pressure conditions in a lab-scale glass reactor to obtain an oligomer with a composition close to the commercial product. In case of poly(ethylene), a small fraction of the ethylene monomer needed to be substituted by an alternative monomer to introduce the  $^{14}\text{C}$ -label while preserving the polymer properties, because  $^{14}\text{C}$ -labelled ethylene is neither available nor suited for synthesis in a radioactive laboratory due to its physico-chemical properties.

$^{14}\text{C}$ -radiolabelling offers a powerful option in the currently very challenging assessment of the environmental fate of polymers. The presentation intends to encourage industry and regulators to consider this technique for future testing of technically relevant polymers.

### 3.07.P-Th308 Assessing the biodegradation of polymers using $^{13}\text{C}$ -labelling

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Polymers play a critical role in many applications to ensure the efficiency, performance, and sustainability of these applications. For applications in which polymers are used directly in the open environment (e.g., agricultural applications such as mulch films and seed coatings), polymer biodegradability is a key sustainable and benign-by-design criterion: biodegradability ensures that microorganisms naturally occurring in a specific receiving environment can metabolically utilize the polymer as a substrate and completely convert the polymer carbon to carbon dioxide ( $\text{CO}_2$ ) and new microbial biomass. As such, biodegradability ensures that biodegradable polymers do not persist in the environment and hence do not accumulate – in contrast to the stable conventional counterparts. Assessing the biodegradability performance of existing biodegradable polymers as well as conceptualizing and designing future biodegradable polymers requires a fundamental understanding of polymer biodegradation in specific receiving environments. In this contribution, we highlight the unique capabilities of using stable carbon isotope ( $^{13}\text{C}$ )-labelled polymers in advancing mechanistic insights into the process of polymer biodegradation. First and foremost, the use of  $^{13}\text{C}$ -labelled polymers in incubations allows selective tracking of polymer carbon mineralization into  $^{13}\text{CO}_2$  and thereby delineating it from  $^{12}\text{CO}_2$  formed through co-occurring mineralization of natural substrates in the incubation vessels. Second, elemental analysis (i.e., combustion) of the incubation medium (e.g., soil) at the end of the incubation coupled to isotope ratio mass spectrometry allows quantifying non-mineralized polymer-added  $^{13}\text{C}$  as  $^{13}\text{CO}_2$  and, thereby, closing the mass balance on polymer  $^{13}\text{C}$  over the course of the incubation. Assessing mass balances is difficult if not impossible when using unlabeled polymers. Finally, using  $^{13}\text{C}$  polymers opens the possibility to directly demonstrate incorporation of polymer  $^{13}\text{C}$  into microbial biomass. These unique advantages of using stable carbon isotope labelling, particularly when combined with solvent-based extractions and quantification of residual biodegradable polymer over the course of the incubation, will be demonstrated by case examples, including the biodegradation of structural polyesters in soils.

### 3.07.P-Th309 Assessing the Suitability of Standardised Tests to Monitor Polymer Biodegradation in Soil

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Interest in, and use of, biodegradable polymers is increasing due to regulatory restrictions on the use of conventional polymers that result in micro/nano plastic production. Increasing amounts of biodegradable polymers will likely be used in soil applications (e.g., plastic mulching films, controlled release fertilisers, and plant protection product formulations). International standard specifications are required for assessing the biodegradability of plastics in soils, coupled with clear and robust standard test methods to verify complete biodegradation within a specific timeframe under controlled environmental conditions.

Current standardised tests focus on respirometric methods, where mineralisation is the endpoint measured by either quantifying evolved carbon dioxide ( $\text{CO}_2$ ) or the biological oxygen ( $\text{O}_2$ ) demand. The suitability of the standard ISO 17556 for determining the biodegradation of biodegradable polymers in soils is investigated using positive control reference materials cellulose and poly(*r*)-3-hydroxybutyrate (PHB). Biodegradation was measured in parallel experimental set-ups that compared  $\text{CO}_2$  evolution using a standard flow through system and  $\text{O}_2$  demand utilising an OxiTop®-IDS system. To determine the reproducibility of the test method across soil types, cellulose degradation was compared in three different soils; two natural soils and an artificial soil (constituents specified in ISO 17556).

Data from both approaches is compared, with results showing clear variability in measured biodegradation between soil types and the measurement methods for both reference materials. Mineralisation measures the ultimate biodegradation endpoint, but the variability seen across soil types and approaches highlights the knowledge gap in understanding how the carbon in the polymer is transformed to carbon in  $\text{CO}_2$ .

This significant knowledge gap in identifying and quantifying the residual polymer and any transformation products, means additional analysis is needed to better understand polymer biodegradation in soil. Pyrolysis gas chromatography mass spectrometry was trialled as an analytical method to quantify residual polymer in soil samples following density-based

extraction from the soil matrix. Initial findings on the suitability of the method for non-labelled biodegradable polymers will be discussed.

### **3.07.P-Th310 Long-term field degradation of mulch films and their effect on soil microbial communities**

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The use of agricultural plastic is increasing each year, and plastic mulch films have been adopted in an agricultural setting worldwide due to their many benefits. However, the removal and subsequent disposal of these plastic films can be challenging, and continued application to the soil surface can result in an accumulation of legacy plastic in soil. This has led to a shift in the agricultural industry towards degradable alternatives. To be classed as biodegradable,  $\geq 90\%$  of the polymer needs to degrade within 24 months, however, it has been shown that biodegradable mulch films often exceed these guidelines in natural environments. Microbial communities in soil are recognised to play an integral role in soil functioning and it has been shown that their composition is highly influenced by soil conditions and biodegradable polymer composition, and that changes in the microbial community can be related to the biodegradation process. In this field-based study, we therefore aimed to investigate the abiotic and biotic degradation rates of one conventional low-density polyethylene mulch film and seven different biodegradable mulch film blends over 1 year, whilst simultaneously assessing their microplastic production rate and effect on the soil bacterial and fungal community. To assess abiotic degradation under natural conditions, mulch films were laid out onto an agricultural field and sampled monthly. Biotic degradation of mulch films in soil was investigated by burying squares of each mulch film in mesh bags in the soil and recovering them over time. To determine if interactions with macrofauna play a role in the fragmentation and degradation of mulch films in soil, two different mesh sizes were used – allowing for only microfauna, and micro-, meso-, and macrofauna interaction. Measurements included changes to the chemical and physical surface structure of the mulch films, potential microplastic generation in the surrounding soil, and effects on microbial community composition. Our results suggest that although changes to chemical and physical surface structure of the films were detected, fragmentation of mulch films buried in soil does not occur within 12 months without additional mechanical stress. These results are highly relevant in our understanding of the difference between fragmentation and degradation, and suggest that degradation of biodegradable mulch films in a natural environment will exceed the current classification standards.

### **3.07.P-Th311 Developing New Methods for Assessing Freshwater Biodegradability of Plastics**

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Biodegradability of plastics is a material property which can be beneficial for selected applications enabling a circular use of resources and avoiding the accumulation of persistent microplastics. Examples of applications are certified compostable organic waste bags or certified soil biodegradable mulch films. The environmental biodegradation of plastics depends on several factors, such as microbial concentration and community composition. The development of new biodegradable plastics requires reliable biodegradation test methods. However, there is a lack of standardized methods for assessing the biodegradability of plastics using freshwater samples, e.g., from lakes and rivers. This study aims to fill this gap by developing new freshwater biodegradation test methods that might be in the future proposed for standardization.

In this work, we developed three new respirometric test methods adapted from ISO test methods used to investigate the biodegradation of plastics exposed to marine inoculum (ISO 23977-1 and ISO 19679): a sediment-freshwater test, a freshwater test and a freshwater test applying an increased microbial concentration factor, adapted from published methods. The polymeric test materials investigated include cellulose, which is ubiquitously available and biodegradable, non-biodegradable low-density polyethylene, and polybutylene sebacate-co-terephthalate (PBSeT). The sediment-freshwater test resulted in significantly different biodegradation of cellulose and PBSeT depending on the environmental sample source used. The freshwater test showed slow biodegradation of cellulose with less than 60% in 180 days and high standard deviations between replicates ( $> \pm 15\%$ ), which would not meet validity criteria of marine standards. The freshwater test with increased microbial concentration showed a more rapid biodegradation of cellulose and PBSeT with  $>95\%$  in 90 days and less deviation between replicates ( $< \pm 5\%$ ).

This study confirms the high potential of methods using inocula with increased microbial concentration to reduce the variability of biodegradation test results. These methods should be considered for standardization and regulation to better reflect natural microbial diversity and minimize false negative test outcomes which could inhibit the development of new biodegradable materials. Methods using sediments require further optimization to reduce the impact of the inoculum composition and thus the variability of biodegradation test results.

### **3.07.P-Th312 Comparison of the characteristics of In-situ Freshwater biofilm development on bioplastics and petrochemical plastics**

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The widespread pollution caused by petroleum-based plastics has spurred the growth of biodegradable bioplastics made from renewable sources. Polylactic acid (PLA) is a prevalent bioplastic and commonly seen as capable of biodegrading in specific

settings. Nevertheless, PLA does not fully biodegrade, thus adding to the problem of microplastic pollution. This study investigates how PLA and Low-density polyethylene (LDPE) would behave differently when exposed to the In-situ freshwater environment at timepoints 14 days and 30 days. Scanning electron microscopy was used to analyze morphology of the biofilm developed. Biofilm was further visualized and quantified with the assistance of Confocal Laser Scanning Microscopy. Distinct Fluorochromes signifying the live and dead biofilm volume confirmed the increase of biofilm volume in both PLA and LDPE. It was observed that phototrophs were much higher on PLA than on LDPE at both time points. Taxonomic analysis by Whole Genome Sequencing indicated distinct and unique plastisphere communities on both PLA and LDPE after 14-day exposure. Microbial genera involved in xenobiotic compound degradation, organic acid cycling and having oxidase and catalase activity was also found. The present study gives us the firsthand biofilm characteristics of plastics of different origins. It could eventually be a step towards extending our understanding of the environmental implication of so-called Bioplastics.

### **3.07.P-Th313 Evaluating Bioplastic Degradation and Fragmentation Using Rainfall Simulation**

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Plastic accumulation in the open environment due to mismanagement has led to growing concern over its pollution and degradation of ecosystems and impacts on human health. The current linear consumption of conventional plastics is widely considered unsustainable. Biodegradable polymers (bioplastics) have been growing in production and use and are frequently marketed as sustainable alternatives to conventional plastics. However, the environmental impacts resulting from the mismanagement or improper disposal of bioplastics is an area of research that is still in its infancy. This is especially true in the context of Ireland, which generates the highest amount of plastic packaging waste in the EU. The expected growth in bioplastic consumption and the lack of bioplastic degradation knowledge coupled with Ireland's high plastic waste generation and abundance of rain serves as the foundation to this research. This research aims to evaluate the degradation and fragmentation of degradable and nondegradable bioplastics in a controlled environment, simulating maritime conditions to gain a better understanding of their possible degradation in an open environment due to mismanagement or littering. Rainfall simulators can replicate natural rainfall events and provide meaningful, quantifiable data. Rainfall simulation and UV ageing will be used under controlled laboratory conditions to analyse bioplastic degradation over time. Rainfall runoff will be collected and analysed for microplastic persistence to glean insight into possible impacts to environmental receptors such as soil and waterways. Following the rainfall simulation, mechanical testing in the form of tensile strength tests will be performed to further analyse the degradation. Lastly, computational modelling and soil burial studies will act as supports to the laboratory testing. To our knowledge, this is the first study of its kind using rainfall simulation to evaluate bioplastic degradation and this research has the potential to offer a novel method for evaluating bioplastic degradation in a simulated oceanic climate environment.

### **3.07.P-Th314 Fragmentation of biodegradable polymers: Assessing and modeling the interim fragmentation in different environmental compartments**

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For specific applications, where plastics cannot be collected in its entirety and recycled, biodegradable polymers can serve as an alternative leaving no persistent microplastics behind, assuring that biodegradation leads to complete mineralization of the polymer carbon into CO<sub>2</sub> and biomass. The loss of the structural integrity is an inevitable part of the biodegradation process and leads to an *interim* formation of fragments.

Biodegradation of polymers is not a homogeneous process, and several factors are influencing the fragmentation of biodegradable polymers, like e.g., microbial colonization, the polymer (blend) composition and shape. The formation of defects, "peninsula" structures and increasing embrittlement during biodegradation leads to interim fragment release. To gain a mechanistic understanding of copolymer blend fragmentation and biodegradation, we studied several biodegradable copolymer blends under industrial composting conditions (ISO 14855), as well as a biodegradable mulch film in soil (ISO 17556). Using different validated extraction techniques for fragments of biodegradable plastics from compost (homogenization, Fenton oxidation, density separation) and soil (centrifugation), Raman as well as fluorescence microscopy analysis was applied to monitor fragment number, shape, size, and identity during biodegradation. The generated data was used for parameterization of the open-source FRAGMENT-MNP model. To understand the chemical and physical mechanisms during fragmentation, we used scanning electron microscopy (SEM) to compare the phase separation in different blends and to determine the area between formed cracks on the surface of polymer films for comparison to Raman/Fluorescence results. In addition, we determined the molar mass distribution at different biodegradation timepoints and assessed the ratio of hydrolyzed polymer to remaining polymer.

The combination of these methods allows to compare fragmentation mechanisms of different types of copolymer blends and helps to predict the timepoint where the total particle counts of different size classes peak during biodegradation. The previously formed fragments will further degrade until mineralized into CO<sub>2</sub> and biomass. Modelling approaches enable the prediction of half-lives of interim fragments, which will depend on the polymer type and environmental conditions.

### 3.07.P-Th315 The Fate of “Compostable” Plastic Bags in Municipal Composting Plants

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Compostable plastic bags are commonly used in Europe for the purpose of collecting organic waste, and are certified to degrade under specific conditions found in industrial composting plants. According to the standard EN 13432 a material is compostable if less than 10% of the original mass remains after 12 weeks of composting and sieving through a 2 mm mesh; at least 90% of the organic material has been converted into CO<sub>2</sub> after 6 months and the material has no negative effects on plant growth. It does not, however, consider realistic composting times in municipal biowaste-treatment plants (Germany 6-9 weeks), nor what happens to fragments below 2 mm in size.

In a consortium project, we distributed three different kinds of biowaste-collection bags made of compostable plastic to citizens of three different municipalities. During the project, we analysed the input and fate of these bags in three different municipal biowaste treatment plants. The aims of the project were:

- To understand the fragmentation and degradation behaviour of the compostable bags throughout the stages in the treatment plants.
- To assess the degradation process of pristine and composted fragments of the compostable bags in soils.
- To survey the attitude, expectations and experiences of the citizens regarding the use of the distributed bags.

The study showed that the majority of compostable plastic bags disintegrated into fragments smaller than 2 mm well before the end of the composting process. However, it also showed that on average, 88% of the microplastics (<2 mm) found in the finished compost were derived from polybutylene adipate terephthalate (PBAT) - the main constituent of the introduced compostable plastic bags. Additionally, degradation tests in soils showed that the mineralisation rate of the composted material was approximately 25% in 145 days under optimal soil conditions, less than that of paper, which had a mineralisation rate of 46% in 145 days.

The distribution of free compostable plastic bags to the municipalities did not result in a visible reduction in the amount of conventional plastics in the incoming biowaste, nor was an increase in biowaste collection observed. Under the aspects of a circular economy, we question the usefulness of compostable biowaste collection bags, as there is a risk of releasing higher amounts of microplastics with potentially long mineralization rates and no obvious benefits to the biowaste stream and compost quality.

### 3.07.P-Th316 Degradation of Biodegradable Plastics

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Decades of extensive production and use of conventional plastics have led to their accumulation in the environment. Bioplastics (bio-based and/or biodegradable) have been promoted as a more sustainable alternative. However, the literature reveals conflicting conclusions regarding their suitability and environmental impact. A primary area of dispute concerns their biodegradability and the conditions necessary for proper degradation. In real-world settings, e.g., composting or marine environments, biodegradable plastics may not degrade as rapidly or efficiently as laboratory tests indicate. Here, we describe two activities that were conducted to address these issues: 1) a systematic literature review to explore the current level of knowledge regarding biodegradable plastics' environmental fate and consequences and 2) a degradation test of commercial biodegradable plastics under simulated industrial composting conditions. The findings of both will be presented and discussed. The review covered the degradation of biodegradable plastic in waste management environments (e.g., compost, sludge, or landfill) and the open environment (e.g., seawater, freshwater, or soil). The results highlight challenges in comparing and quantitatively analyzing data on plastic degradation due to methodological variations, including differences in testing methods, materials, and quantification strategies. Moreover, several research gaps and limitations exist. Notably, there is a need to intensify research on polyhydroxyalkanoates (PHAs), polybutylene adipate terephthalate (PBAT), and polybutylene succinate (PBS) to match the level of polylactic acid (PLA) and starch-based plastics. Research on commercial plastic products must also be intensified. To help bridge these knowledge gaps, a degradation test was carried out on commercially available biodegradable plastics under simulated industrial composting conditions according to ISO 20200. The results indicated disintegration degrees between 75-100 % for all tested biodegradable plastic products, with five of ten reaching complete disintegration within 90 days. The outcomes of both studies offer valuable insights, particularly in guiding discussions about the prospective role of biodegradable plastics within society. Based on our findings, essential knowledge and data gaps will be highlighted, and suggestions will be provided regarding the direction of future research to assess their role as alternatives to conventional plastics.



### 3.07.P-Th317 Plastic Degradation In Natural Seawater Conditions

*Cynthia Gómez, Ricardo Beiras and Sara López Ibáñez, University of Vigo, Spain*

One of the main anthropogenic impacts on the environment is the substantial generation of solid waste that ultimately finds its way into the oceans. Plastics account for 60% to 80% of these residues and their abundance, persistence and chemical composition pose certain risks to marine habitats. In response, biodegradable plastics, commonly referred to as "bioplastics," have emerged as an alternative to traditional plastics, gaining prominence in the market.

Although biodegradable plastics alone cannot fully resolve the issue of plastic littering, which necessitates a fundamental shift in consumer behavior regarding intentional disposal, their use may play a pivotal role in mitigating plastic pollution in cases of accidental or unintended release into the natural environment. Despite their potential, there is limited research on the actual impact and biodegradability of these materials in seawater.

Hence, this study aims to contribute insights into this process. Tests were conducted to assess the fragmentation of PBSeT in comparison to two reference materials, LDPE and cellulose, in two distinct aquatic environments: an equatorial mangrove near the Amazon River mouth (Brazil) and a temperate coastal area in the North Atlantic Ocean (Ría de Vigo, Spain) under benthic and pelagic conditions. At intervals of 5, 28, 60 90 and 120 days, samples from the exposed materials were removed to assess degradation and toxicity through sea urchin embryo tests (SET).

After the initial 28 days, cellulose underwent fragmentation in both habitats (pelagic and benthic) and both exposure environments (South Atlantic equatorial mangrove and North Atlantic temperate estuary ria). Complete disintegration of cellulose occurred after 60 days in both habitats and exposure environments. In contrast, polyethylene films remained intact throughout the entire experiment in both habitats and both exposure environments. Whilst, PBSeT film took a minimum of 60 days to initiate fragmentation in both habitats and both exposure environments. By day 90, PBSeT had completely disintegrated in both habitats in the South Atlantic equatorial mangrove and in the pelagic habitat in the North Atlantic temperate estuary ria. However, under benthic conditions in the North Atlantic temperate estuary ria, a few fragments of PBSeT were still observable after 120 days of exposure. Regarding toxicity, PBSeT exhibited a slight initial toxicity before exposure which dissipated during the initial days.

### 3.07.P-Th318 Bioplastic Bags Degradation Under Marine Conditions In A Mesocosm Facility

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Plastic versatility has promoted its wide use around the globe for all types of applications, what has led to the current unsustainable production levels of these materials. Eventually, they end up in the environment, due to a deficient waste management, and plastic bags are one of the most prevalent products found in marine litter. Apart from persistence and visual nuisance, plastics can pose a risk to marine organisms through toxicity, ingestion and entanglement.

Alternatives to conventional plastics have been created (bioplastics), although it remains to be seen whether these materials are totally successful. In order to avoid entanglement and ingestion, for instance, there is no need of a complete degradation of the product, but rather a decay of its mechanical properties, which are useful to measure as an ecologically relevant endpoint. Also, toxicity along degradation should be also assessed to improve the understanding of these processes, so in this work we propose a method at mesocosm level to test those endpoints.

In this study we test mid-term (4 months) exposures in a mesocosm facility of three plastic bags of different nature (industrial compostable, home compostable and conventional polyethylene) and under different conditions (natural light/darkness, additional inoculum/only natural water). The endpoints measured are toxicity (using the sea urchin embryo test or SET), weight loss and mechanical properties such as tensile strength, deformation at break point and strength at break point. Results

No differences between the treatment with extra inoculum and with no inoculum added were found. Polyethylene bags remained intact along exposure. Weight decreased especially under dark conditions, as well as strength at break point. Also, a slight increase in toxicity was observed in the dark treatment, the same promoting the fastest degradation. Strength at break was selected as the most suitable endpoint for mechanical properties.

This approach is a relatively fast method to assess plastic degradation in conditions representative of the marine environment. Bio and conventional plastics show clear differences in the evolution of toxicity and degradation processes. Lower variability, high ecological relevance and the standardizations available for strength at break point are the reasons why it is recommended for study mechanical decay. This kind of works are useful to help classifying new plastics products according to their marine impact and behaviour.

### 3.07.P-Th319 Investigations into Ageing of Monofilaments Under Various Test Conditions in the Sea

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Problems associated with marine plastic litter caused by the fisheries and aquaculture sectors can be significantly reduced if traditional plastics like Polyamide 6 (PA, Nylon) are replaced by new biodegradable materials. Materials designed to be biodegradable, such as polybutylene succinate co-adipate-co-terephthalate (PBSAT) are intended to degrade or decompose after a certain period in water and thereby lose their ghost fishing capacity more quickly than conventional gear. The materials should be user-friendly during the service life with a short (and non-linear) degradation phase afterwards. In addition to degrade more rapidly, such materials should have stable mechanical properties during the period of use. Previous small-scale tests have however shown that further research and industrial development is necessary for the commercialization and use of biodegradable plastics in these ocean-based industries.

Here, a framework for testing of biodegradability and environmental impacts have been developed. Laboratory and field testing of biodegradable fishing lines (monofilaments) are carried out in conditions representing different marine conditions. The degradation and physical and chemical integrity of assumed biodegradable and conventional lines is evaluated during an extended test period (up to 5 years), starting in 2021. In the field, marine biodegradation of lines (PBSAT and non-degradable PA as the control) are tested *in situ* in different marine habitats (Skagerrak Sea, North Sea, Baltic Sea, Adriatic Sea, and Norwegian Sea) to cover a wide temperature range. Additionally, laboratory experiments are performed where UV-exposed materials (weathering) are included in the setup, allowing for testing of 'aged' materials. The physical-, chemical-, and microbial- degradation is quantified by a suite of tests such as mechanical properties, SEM, py-GC-MS, respirometric analysis and microbial community analysis.

The long-term study of polymers designed to be biodegradable and traditional polymers will contribute to our future understanding of the degradation of polymers in the sea and will help the fishing industry develop more sustainable innovations for the global market.

### **3.07.P-Th320 Laccases as a Biocatalytic Tool for the Degradation of Biodegradable Plastics**

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Due to the constantly increasing production of plastics, which amounts to around 400 million tons per year, the pollution of the environment by plastic waste is increasing. Biodegradable materials are seen as a solution to this problem. However, it must be noted that little is known about the fate of biodegradable polymers (BPs) in the environment and therefore it is necessary to decipher these processes. Polycaprolactones (PCL) are semi-crystalline aliphatic polyesters. Besides their classical purposes for packaging materials, it is used to produce controlled-release drugs due to their slow biodegradability.

It has meanwhile been shown that PCL microplastics can also be found in the terrestrial and marine environment, indicating that PCL is also not easily degradable under natural conditions. The literature repeatedly describes examples of how fungi, bacteria, or microbial communities are involved in degrading synthetic and biodegradable polymers.

In order to gain a more detailed insight, the possible involvement of the fungal enzyme laccase (EC 1.10.3.2) in synthetic polymer decomposition was tested. Laccases belonging to the class of oxidoreductases and have already been used for the degradation processes of various environmental pollutants.

Our results indicate that the oxidoreductase laccase can oxidatively attack various polycaprolactones. More detailed insights via UPLC-ELSD and UPLC-HRMS/SFC are in support of a significant influence of PCL structures on the degradation mechanism and their rate. It was found that diethylene glycol-based polycaprolactone offers an excellent target for laccase attack seemingly due to the diethylene glycol. UPLC-HRMS analyses have shown that the diethylene glycol is oxidatively cleaved in the first step of the reaction. It could also be demonstrated that oxidative degradation must proceed differently for PCLs with different structures, some of which are not diethylene glycol-initiated.

Therefore, another starting point for oxidative PCL degradation occurs. In our investigations, various transformation products (TPs) were detected, related structures were proposed, and their stability under the applied conditions was checked. The knowledge of the degradation pathways and stable TPs is essential for the discussion on the use of these biodegradable polymers and the anthropogenic footprint, as BPs are seen by society as the optimal and simple solution to tackle the plastic pollution problem.

### **3.08 Chemical Fate in the Soil-Plant System and Evaluation of Related Impacts and Risks**

#### **3.08.T-01 Soil sorption and plant uptake of five antibiotics by spinach (*Spinacia oleracea*) and radish (*Raphanus sativus*)**

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Antibiotic residues are widely detected in agricultural soils and animal manure fertilizers. Once in agricultural lands, the fate of antibiotics depends on the physiochemical properties of the compounds, soil sorption potential, crop species and environmental conditions. In this study we investigated the fate and distribution of five co-applied antibiotics in soil-pore water-plant system, and their uptake and translocation to spinach (*Spinacia oleracea*) and radish (*Raphanus sativus*). The plants were grown for 6 weeks under controlled environmental conditions in soil spiked with antibiotics (0-10 mg kg<sup>-1</sup> concentration range for individual antibiotics), after which they were harvested. The selected antibiotics, clarithromycin (CLA), sulfamethoxazole (SMZ), trimethoprim (TMP), enrofloxacin (ENR) and chlortetracycline (CTC), represented a range of characteristics, for example with cationic (CLA), anionic (SMZ), and neutral (ENR, CTC, TMP) species dominating in the experimental conditions. Soil and pore water samples were collected at the end of the experiment. Additionally, timepoint samples of soil and pore water were collected at days 0, 2, 14 and 21. The soil and plant samples were extracted with QuEChERS (**Quick, Easy, Cheap, Effective, Rugged, and Safe**) method and analyzed with LC- Triplequad MS/MS. The soil samples collected over the course of the experiment demonstrated different dissipation behavior of the spiked antibiotics. For example, in the 1 mg kg<sup>-1</sup> spike concentration of SMZ, the half-life was calculated to be 3.3 days, whereas for CLA, the concentration remained constant throughout the experiment. The uptake of antibiotics to plants was marginal compared to the total spiked antibiotic load. Nevertheless, antibiotics were observed both in the plant leaves and roots where the uptake and translocation was dependent on both the antibiotic compound and the plant species. For example, in the 1 mg kg<sup>-1</sup> treatment, SMZ was found both in the plant leave and roots fraction, but the observed concentrations were notably higher in spinach compared to the radish (1 to 3 orders of magnitude in leaves and roots, respectively). The observed sorption, dissipation, uptake and distribution patterns in this study can help to understand the varying environmental behavior of antibiotics.

### **3.08.T-02 Evaluating the Translocation of PPCPs into *Zea mays* Following Wastewater Reuse – A Model validation via an Israeli Scenario**

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This research addresses the pathway for Pharmaceuticals and Personal Care Products (PPCPs) to enter terrestrial soils through the reuse of wastewater in agriculture, focusing on *Zea mays*. Recognizing the limitations of current monitoring, which only considers a standard list of contaminants, this research employs a novel model framework, combining dynamic and steady-state systems, to prioritize PPCP contaminants based on their chemical structure alone. The research involved fieldwork and laboratory analyses in Israel's Negav and Hamakim districts, areas known for high rates of wastewater reuse and *Zea mays* cultivation. Corn soil and irrigation water samples were subjected to Solid Phase Extraction and analysis using LC-MS/MS, targeting 72 PPCPs. The Israeli specific model was adjusted to use experimental data derived in Israel, predictive tools were however used to assess PPCPs affinity to plant proteins (KHSA). The study identified 31.94% of screened contaminants in irrigation water and 7.2% in *Zea mays* grain. Key findings include the detection of lamotrigine and benzotriazole in high concentrations in irrigation water, and significant presence of gabapentin, caffeine, carbamazepine, and alprazolam in corn. The model framework successfully predicted 90% of the measured environmental concentrations (MECs), with some discrepancies noted for gabapentin and venlafaxine. Future work includes non-targeted screening using HR-MS/MS to confirm the presence of contaminants and refine the predictive model. The study marks the first report of pharmaceutical translocation into *Zea mays* following wastewater reuse. The Israel-specific model underscores the need for case-specific models in environmental risk assessments of PPCPs. The study validates the use of chemical structure alone for risk assessment, with improvements possible through the integration of experimental data on fate and physicochemical properties.

### **3.08.T-03 Comprehensive approach to model pesticide residues in plants**

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Understanding of the fate of chemicals in plants is crucial to assess and manage pesticide residues in food commodities, with the aim to reduce dietary and environmental risk and to approach a minimum residue situation. Assessment of pesticide residues for regulatory purposes is currently based on experimental studies with few, frequently only one final sampling of plant material. These few measurements require much experimental effort but hardly allow to identify the drivers of residue formation or to explain observations at other sites under different conditions. As a consequence, the dietary safety assessment must currently be based on worst case observed residue concentrations.

In this work we present a mechanistic modelling approach to describe chemical residues in plant considering soil water flow and chemical transport, crop (biomass) growth and chemical translocation processes in plants. Uptake of chemical into the plant is implemented via the roots and via the canopy. Canopy processes include interception, dissipation from foliage, loss to the ground via washoff and penetration of chemical through the cuticula into the plant. Crop growth processes consider the assimilation rate, partitioning to different plant compartments, phenological and biomass development. Coupling of growth with soil water processes allows the consideration of drought and oxygen stress. Finally translocation, partitioning, metabolism and growth dilution of chemicals in the plant is described using established plant compartment model approaches. Both transport systems in plant, xylem and phloem, are considered.

The current implementation uses SWAP and PEARL which are state-of-the-art 1-D soil water flow and transport models. This choice allowed to use detailed WOFOST crop growth modules coupled to SWAP. Results of the soil and growth models are

stored in a specific interface which serves as input for the plant translocation model. The use of such an interface allows the use of other soil and growth models or experimentally measured data directly.

Model runs with exemplary scenarios demonstrated the functionality of the model approach and the plausibility of the model results. Next step is the application to experimental studies to explore means of parameterisation and the necessity of calibration. Currently the model is limited to the description of neutral chemicals. Specific features of dissociating chemicals (e.g. ion trapping) are foreseen to be implemented in future.

### **3.08.T-04 Positive Impacts of Doxycycline on Mycorrhizal Structure and Function in the Agro-Environment**

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As the world's population and demand for food increases, soil is being degraded and crop production is slowing due to unsustainable agriculture practices. Synthetic fertilisers are used to supplement plant nutrition in conventional agriculture, and these are reliant on energy-intensive production processes and/or finite natural resources. These products are common air, land, and water pollutants, and their long-term use can reduce soil quality and diversity. Manure application is a good way to improve soil quality and, in turn, crop production, helping move away from synthetic fertiliser use. Treating soils with manure can improve soil health by supplementing soil organic matter, improving soil structure, and increasing microbial biomass, including arbuscular mycorrhizal (AM) fungi. However, veterinary pharmaceutical contaminants have been found in manures and soils after manure application. Here, we focus on the antibiotic, doxycycline. The inadvertent introduction of doxycycline to soils could negatively impact soil health, specifically AM fungi, a group of symbiotic soil fungi that significantly contribute to soil nutrient cycling and plant nutrition. Using radioisotope tracers to investigate the effect of doxycycline on the structure and function of AM fungi, we tracked carbon-for-phosphorus (C-for-P) exchange between AM fungi and dwarf runner beans exposed to environmentally relevant concentrations of doxycycline. We found that doxycycline increased AM hyphal density in soils but had no impact on root colonisation by AM fungi. Nutritionally, doxycycline increased plant assimilation of AM-acquired P, while the total amount of P in shoot tissues was unchanged. Mirroring the improved P assimilation, AM fungi assimilated a greater amount of plant-fixed C when they were exposed to doxycycline. Our results suggest that veterinary doxycycline alone improves AM structure and function. However, as doxycycline is a broad-spectrum antibiotic, there is potential for it to negatively affect beneficial soil bacteria, which could result in overall negative ecosystem impacts.

### **3.08.T-05 Assessing the potential risks of organic lettuce cultivated in an area irrigated with reclaimed water**

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Water scarcity is becoming more frequent principally as the result of longer drought periods, changes in socio-economic factors and the increase in demographic growth. In this context, circular economy routes for a sustainable use of water are currently arising, especially in the agricultural sector, in which reclaimed water is used for irrigation of crops and at the same time replenishes the aquifer. Despite these efforts, reclaimed water has been found to introduce contaminants of emerging concern (CECs) into the ecosystem, because of that, is needed to assess their prevalence, potential environmental and human health effects and to establish regulations controlling their presence. This study aimed to investigate the potential risk of organic lettuce cultivated in an agricultural area whose irrigation system incorporates reclaimed water. The objectives of this work were (1) to optimize and validate a QuEChERS extraction method for the LC-MS/MS analysis in lettuce of some selected CECs found to be present and potentially toxic for the aquatic environment in the water used for irrigation in the area of study (2) to evaluate potential differences in their occurrence between washed and unwashed lettuce, and (3) to assess their associated risk for the human health. For these purpose, QuEChERS extraction procedure was optimized through a 2<sup>6-2</sup> fractional factorial design of experiments and the screening was performed using a UPLC-QTOF-MS/HRMS. Forty-three targeted CECs were monitored in wash and not-wash lettuce samples. The samples were obtained from a single organic planting plot from the Baix Llobregat Agrarian Park (Catalonia), an agricultural area influenced by the addition of reclaimed water in the irrigation system. Results showed that 10 out of the 43 targeted compounds were detected and quantified in at least one of the 10 lettuce samples with average values ranging between 0.1 and 71.1 ng/g. Dibutyl phthalate, diethyl phthalate and triethyl phosphate presented concentrations exceeding the MRL considered (10ng/g), indicating a potential risk to human health. The compounds found in higher concentrations were some phthalates and phosphates, commonly used as industrial chemicals. Considering all present CECs, no differences were observed between the concentrations found in WL and NWL. The concentration of certain identified compounds showed a significant correlation, suggesting that their uses and uptake in lettuce are similar.

### **3.08.P Chemical Fate in the Soil-Plant System and Evaluation of Related Impacts and Risks**

### **3.08.P-Tu178 Organic chemical contaminants in fertilizers, amended soils, and corns**

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Fertilizing residual materials (FRMs) from industrial or municipal origins are used to enhance soil fertility and considered valuable sources of nutrients for field crops. The dispersion of FRMs in agricultural soils however raises concerns about the potential chemical risks associated with emerging contaminants including bisphenols and plasticizers. To better understand the fate of these chemicals, the current study aimed at developing analytical methods for the simultaneous targeted and non-targeted analysis of contaminants in various types of fertilizers (including FRMs), amended soils, and crop plant tissues. The methods were applied to samples obtained under real controlled field conditions in Canada.

More specifically, FRM samples (n=8 types), one cattle manure, three mineral fertilizers, amended soils, and corns were obtained from two experimental fields in Quebec, Canada. After freeze-drying and homogenization, samples were extracted using an ultrasound-based method and analyzed by LC-QToF-MS. Analytical methods were validated for absolute recoveries, matrix effects, method detection limits (MDLs), and precision using a certified reference material (SRM 2781) and three FRM matrices (municipal biosolids, deinking biosolids, and ash).

The extraction methods showed overall satisfactory performances and were applied to real samples. Municipal biosolids, deinking biosolids, digestate of biosolids, and lagoon biosolids exhibited relatively high levels of bisphenols/plasticizers compared to other fertilizer samples. Deinking biosolids were notably contaminated by bisphenol S (BPS) and other related compounds such as TGSA, D-8, and D-90. The non-targeted workflow effectively identified over 40 contaminants from diverse families in the municipal biosolids and deinking biosolids. To the best of our knowledge, many contaminants revealed through this novel workflow (e.g. tetradecylamine, *p*-toluenesulfonamide, 4-dodecylbenzenesulfonic acid, Cyanox 2246, triethylene glycol monobutyl ether, and pinosylvin methyl ether) had never been reported in FRMs, demonstrating the need to include non-targeted analysis in the surveillance of FRMs.

The presence of bisphenols (BPA, BPS, BPAF, BPBP, D-8, D-90, and TGSA) and phthalate-related plasticizers (DBP, DiBP, BBzP, DEHP, DPHP, and DiNP) was confirmed in soils after fertilizers application, at concentrations below 50 ng g<sup>-1</sup> dw. Additionally, the levels of targeted bisphenols and plasticizers were monitored in corn.

### **3.08.P-Tu179 Fate of Hormones, Pharmaceuticals and PFAS in the Soil-Plant System and Implications for Food Safety**

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A key requirement for a circular bio-economy is that by-products, e.g. manure, urine, sewage sludge and paper sludge are returned to soil for replenishing nutrients for crops. However, a major concern is that these by-products contain chemicals that are hazardous for humans, animals and the environment, such as hormones, pharmaceuticals and PFAS (per and polyfluoralkyl substances). Recently, a case in Germany showed that application of PFAS-contaminated paper sludge led to widespread contamination of soils. In the Netherlands, PFAS were found in soils and crops in allotment gardens close to fluorochemical production facilities, leading to the advice not to eat the crops from gardens surrounding those places.

Pollution of the soil by chemical hazards may lead to uptake of pollutants by the crops growing on those soils. The here-presented work contains a tiered strategy to assess the fate of chemical hazards in soil, considering persistence, mobility and degradation products with their activity. This strategy was applied to relevant chemicals (hormones, pharmaceuticals, PFAS) in characterized soils. Moreover, we studied the uptake of these substances from soil into plants, including uptake over time, and their subsequent translocation to the edible parts. It was proven that indeed hormones, antibiotics and PFAS are taken up by crops. Assessing uniform uptake rates of chemical food safety hazards by crops is a challenging task, as the total chemical uptake by the crops and respective translocation into edible plant parts may be highly variable, and dependent on many different factors. As such, in the mentioned experimental study, a differentiation in the translocation pattern was found between chemicals, between a leafy crop and a root crop and over time. In addition, other factors have been reported influencing chemical uptake, such as soil biotic and abiotic characteristics, climate, growing conditions and the presence of other chemicals.

Quantitative knowledge on how these different factors in the soil-plant system relate to each other and what is most determining under which circumstances, is still largely lacking. It is essential, from a food and environmental safety perspective, to increase our insights in the crucial soil-plant processes, in order to assess food safety risks and facilitate the assessment of human and environmental exposure. The authors invite the audience to exchange knowledge and ideas.

### **3.08.P-Tu180 Pharmaceuticals in a Soil-Plant System Irrigated with Reclaimed Water in the Mediterranean Area: Environmental and Human Health Risk Assessment.**

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Reuse of wastewater is a strategy to cope with water scarcity and the urgent need of alternative freshwater sources in Mediterranean Areas. The (re)use of reclaimed water for agriculture purposes offers many benefits but careful attention needs to be paid to the presence of pathogens, suspended solids, and regulated and non-regulated contaminants in the irrigation water after the polishing treatments.

The objective of this study was to prioritise the pharmaceuticals (among the most studied emerging pollutants) that might pose a risk to environment and human health when reclaimed water is used for irrigation of crops, in particular for lettuce and tomato plants. A comprehensive study was conducted to first identify the pharmaceuticals frequently detected in secondary effluent in Catalonia (NE Spain) of various therapeutic classes, such as analgesics and anti-inflammatories, antibiotics,  $\beta$ -blockers, and psychiatric drugs. A priority list of pharmaceuticals was established, based not only on their occurrence in secondary effluents (O) (real data of 10 years of monitoring), but also on persistence in terms of removal in conventional treatments (P), bioaccumulation potential (B), toxicity for aquatic organisms (T), and risk to terrestrial environment as well as to human health through the consumption of crops.

The findings revealed that 6 pharmaceuticals (especially iopromide) could pose a risk to the organisms living in the soil irrigated with reclaimed water and up to 7 pharmaceuticals (especially carbamazepine and its transformation products) were potentially taken up by the crops. Nonetheless, none was found at concentrations that could pose a risk for human consumption. The study highlights the need to accurately select the pharmaceutical residues to be included in wastewater monitoring programmes as well as polishing treatments to eventually reduce their presence in soils and crops irrigated with reclaimed water.

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### **3.08.P-Tu181 Presence of Pharmaceuticals and Other Emerging Contaminants in Biogenic Matrices Used in Agriculture as Fertilizer and in Soil and Lettuce**

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Sewage sludge is a typical by-product of our society and its reuse in agriculture is potentially an ideal solution for its disposal. The application of sludge and other biogenic matrices on overexploited agricultural land can bring great benefits due to their content of organic carbon, nitrogen, phosphorus and micronutrients. However, great attention must be paid to their potential adverse effects due to the presence of several classes of emerging contaminants (ECs), such as pharmaceuticals, antibiotics, personal care products and perfluorinated compounds. This may result in the transfer of ECs to the crops with potential risks for human health. The aim of this study was to investigate the presence of about 40 ECs in different substrates (sewage sludge, compost, digestate, pig and cow manure) and to assess ECs uptake in lettuce (*Lactuca sativa*) grown in soil mixtures containing the different substrates, as well as in the soil in which the lettuce was grown. The analysis of the ECs was performed by liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The results showed different profiles of contamination in the different substrates investigated, with prevalence of fluoroquinolones, antibiotics for human use, in sewage sludge and of veterinary antibiotics in manure. Some antibiotics and plasticizers were found in soil and *Lactuca sativa* indicating the possible uptake from soils to crops. The discharge of ECs into agricultural lands through the application of biogenic matrices can create a stressful condition for the terrestrial ecosystems threatening its functioning. Moreover, the presence of antibiotics may also promote the spread of antibiotic resistance, a serious threat for human health. This study improved information on ECs presence in sludge and manure and their uptake in lettuce, with the overall goal to convert a potentially risky practice such as the application of biogenic matrices in agriculture into a safe process of circular economy.

### **3.08.P-Tu182 Evaluation of the dynamics and impact of nanomagnetites (Fe<sub>3</sub>O<sub>4</sub>) on lettuce (*Lactuca sativa*) in a context of cadmium contaminated soil**

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Soil contamination by toxic metals such as cadmium (Cd) is a major issue for our society. Over the last few decades, around 22,000 tonnes of Cd have been released into the environment worldwide. For soil remediation, several approaches have been tested. Due to their physicochemical properties and their nanometric size, nanomaterials have been receiving substantial growing attention. Among these nanoparticles, nanomagnetites (Fe<sub>3</sub>O<sub>4</sub>, NPs-Fe) have great potential for soil remediation. Evaluating the impact and fate of these nanoparticles therefore seems essential.

This research aimed to evaluate the effect of nanomagnetites on lettuce (*Lactuca sativa*) development and their role as an adjuvant to improve the phytoextraction efficiency of Cd in soil. In this context, nanomagnetites were applied (1.2 g.kg<sup>-1</sup> soil)

to a Cd contaminated soil (20 mg.kg<sup>-1</sup> soil). Four conditions were tested: control, Cd, NPs-Fe and Cd+NPs-Fe. The plants were harvested, weighed, freeze-dried and analysed by ICP-MS after 46 days of exposure to determine metal concentrations. Photosynthetic yield measurements were also taken before harvesting. The dynamics of nanomagnetites from soil to plants were also studied by measurement of magnetic susceptibility.

The results show a negative impact of Cd and NPs-Fe on lettuce development, with a reduction in biomass of around 50% for each of the treatments alone and over 70% for the Cd+NPs-Fe treatment. While the analyses do not show any difference in Fe content between the 4 treatments, a significant difference is observed for plant Cd content, which is roughly two times higher under NPs-Fe treatments compared to nanomagnetites-untreated conditions. Concerning the photosynthetic yield, a significant difference is only observed between control and Cd+NPs-Fe. Finally, a magnetic signal is detected in plants treated with NPs-Fe (NPs-Fe and Cd+NPs-Fe), indicating the presence of NPs-Fe in plant tissues.

This study showed that at the concentrations tested, nanomagnetites have a negative impact on the development of lettuce plants, but significantly increase the plant Cd concentration. It could be interesting to study the effect of different concentrations of NPs-Fe on lettuce to complete this work.

Keywords : Iron nanoparticles; Magnetite; Pollutants; Plant; Ecotoxicity; Remediation

### **3.08.P-Tu183 Contamination of water, soil, and plants by micropollutants from treated wastewater and sewage sludge: Results from the second-year field experiment**

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An experiment aimed at monitoring the behavior of selected micropollutants in soil-water-plant system was established in the wastewater treatment plant for České Budějovice in the Czech Republic. Here, nine raised beds were installed in March 2021 near the outlet channel. Two beds contain soil taken from the surface horizon of the Arenosol Epieuric developed on sand and seven beds contain soil from the surface horizon of the Haplic Cambisol on paragneiss. Either maize or a mixture of different vegetables was grown in the beds in 2021. Four treatments were carried out for maize grown in the Cambisol beds: A. irrigation with tap water (control); B. irrigation with wastewater; C. application of composted sewage sludge before sowing; and D. application of sewage sludge before sowing. Three treatments A, B and C were suggested for vegetables grown in the Cambisol beds and two treatments A and B were proposed for vegetables grown in the Arenosol beds. Results obtained during this year have been published (<https://doi.org/10.1016/j.scitotenv.2023.167965>). In 2022, two different mixtures of vegetables were grown in these beds. Potatoes, soybeans, and beans were planted in originally maize beds. Kohlrabi, parsley, radishes, and brussels sprouts were planted in the originally vegetable beds. The treatments remained the same with one difference. The beds with the compost were additionally irrigated with wastewater. Composition and average concentrations of compounds in wastewater measured in 2022 were like those in 2021. Similarly, composition and concentrations of compounds in sludge and composted sludge measured in 2022 were like those in 2021. While less compounds leaked from the beds in 2022 than in 2021, a larger spectrum of compounds was quantified in soils in 2022 than in 2021. Taken up and mobile compounds in plants were mostly accumulated in plant leaves. Mostly no compounds were quantified in fruits (e.g., soybean pods). Compounds of low mobility remained in soils and belowground plant tissues. For example, low concentrations were recorded on the surface of potato tubers but not inside. The work was supported by the Ministry of Agriculture of the Czech Republic, projects No. QK21020080 and QK23020018, and the European Structural and Investment Funds projects No. CZ.02.1.01/0.0/0.0/16\_019/0000845.

### **3.08.P-Tu184 Determination of Polycyclic Aromatic Hydrocarbon (PAHs) Associated with Vehicular Emission and its Effect on Air, Soil, and Food Cultivars Planted Along The Major Highways in Enugu State, Nigeria.**

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Air, soil, and vegetables from six farmlands around three selected highways, Enugu-Abakiliki, Enugu-Onitsha, and Eke-Iwollo in Enugu state were investigated for the presence of toxic air gaseous constituents and Polycyclic aromatic hydrocarbon (PAHs). Two of the highways (Enugu-Abakiliki and Enugu-Onitsha) are associated with high vehicular emission due to the high traffic density while Eke-Iwollo highway (control) is associated with low traffic density. Two farmlands (5meters and 500 meters away from the high way) from each location were studied. Polycyclic aromatic hydrocarbon (PAHs) in the soil and vegetables were determined using Gas Chromatography-Mass Spectrophotometer. Air-vehicular emission (NO<sub>2</sub>, SO<sub>2</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>, and total carbon) were monitored morning, afternoon, and evening with Aeroqual/Crowcon Gasman monitors and Garmin GPS. The results of the investigation revealed high levels of the toxic gaseous substances (NO<sub>2</sub>, SO<sub>2</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>) in Enugu-Abakiliki and Enugu-Onitsha farms (5 meters from the highway). It was observed that the mean concentration of the low, medium, high molecular weight PAHs in soils were highly present and above WHO (0.3mg/kg) permissible level in both Enugu-Abakiliki and Enugu-Onitsha farmlands (5 meters away from the high way) but below the detectable limit in other study sites, Vegetables (bitter leaf, pumpkin leaf, and cassava leaf) were equally proportionately contaminated with these low and high molecular weight PAHs in Enugu-Onitsha and Enugu-Abakiliki farmlands (5 meters from the high way). Given the

appreciable accumulation of these toxicants in vegetables, it suggests that food remains a major endogenous source of PAHs, among the people of these areas.

### **3.08.P-Tu185 Fate of Per- and Polyfluoroalkyl Substances (PFAS) from Soil to Edible Crops: Relevance for the Safety of Circular Food Systems**

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Closing agricultural production loops by application of side- and residual streams for food and feed production can help to improve the sustainability of food systems. However, besides the reintroduction of nutrients, this can also result in the (re)introduction or accumulation of hazardous chemicals in the food system. To understand the subsequent possible effects on food safety, quantitative knowledge is needed on the fate of relevant chemical hazards through circular food systems. To increase this understanding, the present study evaluated the transfer of spiked per- and polyfluoroalkyl substances (PFAS) from biosolids-amended soils to edible crops. The aim was to collect experimental data on the uptake and translocation from soil to crops.

Four selected PFAS (i.e., PFOA, PFNA, PFHxS, PFOS) were spiked to sewage- or paper sludge, individually and in mixture. The spiked sludge was subsequently mixed with soil, resulting in final concentrations of 5 or 100 ng/g in the soil for each compound. The spiked biosolid-amended soils were used in a closed pot system to grow radish and greens (a leafy vegetable). After harvesting the crops, roots and shoots were separated and PFAS concentrations were quantified by extraction and clean-up of the samples and analysis by liquid chromatography-tandem mass spectrometry.

The uptake of PFAS was shown for both crops. For radish, higher PFAS concentrations were found in the shoots as compared to the roots, while for the leafy vegetable, higher PFAS concentrations were found in the roots as compared to the shoots. This illustrates the difference between crop types and parts. The PFAS concentrations in the radish roots ranged between 2.2-5.9 ng/g w.w. (exposure concentration 100 ng/g), while the concentrations in the shoots of the leafy vegetable ranged between 0.5-13.4 ng/g w.w. (exposure concentration 100 ng/g). The concentrations of the PFAS in the edible parts of both crops were ordered as follows: PFOA > PFHxS > PFNA > PFOS.

Overall, the fate of PFAS differed between the studied crops and parts. Other factors may play a role in the fate of PFAS from soil to crop as well. Combining in future the here-described approach with other (experimental) test strategies to quantify the impacts of influential factors on the fate of chemical hazards through a circular food system, will be relevant to ensure food safety.

### **3.08.P-Tu186 Uptake of Per- and Polyfluoroalkyl Substances From Soil to Plants – A Meta-Analysis**

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Per- and polyfluoroalkyl substances (PFAS) are a diverse class of industrial chemicals that have been used in various products and processes for over 80 years. Due to their widespread use and persistence, PFAS can be found in all environmental compartments, including groundwater, rainwater, and soil. In addition, many PFAS have been associated with adverse impacts on ecosystems and human health.

Therefore, it is crucial to establish threshold values for PFAS in soil, water, and food to protect humans and animals that are taking up PFAS from these sources. However, in some countries, such as Switzerland, regulatory values have not yet been established or have only been established for certain PFAS. To determine soil guideline values, it is important to consider that soil contamination can result in exposure to PFAS and other chemicals through direct inhalation or ingestion of polluted soil, as well as through consumption of plants grown on contaminated land or animal products. If animals graze on polluted soil or feed on contaminated plants, they can accumulate PFAS, ultimately leading to human exposure if animal products are consumed.

The objective of the present study is to investigate the transfer of PFAS from soil to plants with a particular focus on perfluorinated alkyl carboxylates and sulfonates. This forms part of the groundwork for proposing soil guideline values at a later stage in the project.

To this end, a literature review is conducted, and experimental data are collected into an SQL database. The collected data include information on PFAS concentrations in soils and plants, as well as various experimental factors such as soil and plant features, study duration, study location and experimental design. This comprehensive database enables an in-depth analysis of PFAS uptake patterns. A similar exercise was conducted in 2020, however, more data have become available in the meantime, including data from Rastatt in Germany so that the existing database can be extended to obtain a more comprehensive overview.

Finally, the results will be presented, showing which PFAS are taken up into plants and whether certain plants are more susceptible to PFAS uptake. The collected data will be used to investigate which factors determine PFAS uptake, including



chemical properties (e.g., functional groups, molecular weight), plant characteristics (e.g., compartment, fat content, protein content), and soil characteristics (e.g., organic carbon content, pH).

### **3.08.P-Tu187 Riparian Plant Uptake of Current-Use Pesticides in Small Streams from Vine-Growing dominated River Basins: Aquatic-Terrestrial Linkages**

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Surface water pollution is a widely recognised problem, with chemicals like plant protection products (PPP) ending up e.g. in fluvial system worldwide. Fluvial riparian zones are known as diversity hotspots, and serve as a niche ecosystem to various taxa, with plants like the stinging nettle serving as an important generalist and specialist host plant. As the introduced PPP are expected to interact with the riparian zones, it is hypothesized that non-target riparian plants, such as e.g. the stinging nettle, are exposed to PPPs. One of the proposed transport pathways from surface water contamination to non-target riparian plants is an elevated water level through flooding. This study is investigating flooding as a vector of PPPs into riparian zones with its non-target plants.

Therefore, a steep- and a close-by shallow-slope riverbank in each five streams served as paired study sites to compare frequently flooded areas with non-frequently flooded areas. Sampling root-zone soil and plant individuals from these respective areas allowed to assess the PPPs levels in these matrices and linking it to the flooding intensity. In each of the two areas in these five streams, five individuals of each of five plant species have been sampled. The soil and plant material were extracted using an acetonitrile-based extraction method and PPPs levels for 98 chemicals have been assessed using an UHPLC-ESI-MS/MS complex.

As hypothesized, we found that in frequently flooded areas, plants are more contaminated, qualitatively, and quantitatively compared to the close-by higher riverbank situated plants. These findings are underlined by the concentration levels within the root-zone soil samples, allowing to track the exposure pathway from the surface water and the sediments carried into riparian soil and the subsequent bioaccumulation in the riparian plant systems.

This newly proposed exposure pathway opens another threat for diverse and sensitive riparian ecosystem, which may have carry-on effects on the riparian food web.

### **3.08.P-Tu188 Bio-accumulation of Heavy metals in Crops and Pollution Index Assessment**

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Heavy metal pollution in soil has been concerned because of bioaccumulation in crops and consequently, can cause adverse effect on human health. In this study, heavy metal pollution in soil was evaluated based on Nemerow soil pollution index (NSPI) and bioaccumulation factor (BCF) in paddy soil in Korea. Total of 100 soil sample and rice (*Oryza sativa.*) was collected for heavy metal measurement. In order to evaluate bioavailable fraction of heavy metals in soil, Mehlich 3 extractant was used for heavy metal extraction in soil. Among 3 different heavy metals (As, Cd, Pb), the average concentration of Pb was the highest in both soil (3.9 mg kg<sup>-1</sup>) and crop (0.6 mg kg<sup>-1</sup>) followed by As in soil (1.4 mg kg<sup>-1</sup>) and crop (0.3 mg kg<sup>-1</sup>). Calculated NSPI was ranged 0.04-7.71 with average value of 0.35 indicating that heavy metal pollution in soil was medium to high depending on sampling area. Among 100 crop samples, 26 samples were exceed the threshold value of Pb (0.2 mg kg<sup>-1</sup>) with the highest concentration of 7.58 mg kg<sup>-1</sup> whereas, none of the Cd concentration was exceeded the threshold value (0.2 mg kg<sup>-1</sup>) in rice. In addition, value of BCF was ranged for Pb (2.6 – 15.4 ) and Cd (0.1-0.2 ) and indicating that much higher concentration of Pb can be accumulated in crops compared to Cd. Our result showed that Pb pollution in soil was severe and consequently, accumulation of Pb can be concerned in rice cultivation. Given the potential adverse effects associated with heavy metal consumption, remediation strategies should be adapted to reduce heavy metal concentration in both soil and crops.

### **3.08.P-Tu189 Evaluating Plant Uptake Through a Standard Soil-Based Biotest: Insights from Case Studies on Flame Retardants and Nanoplastics**

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The wide set of experimental conditions performed in published soil-based studies on plant uptake of contaminants make comparisons and data quality assessment challenging. The use of standardized soil-based methods, like ISO 16198, also known as RHIZOtest, could enhance repeatability and data reliability. Considering its acknowledged advantages over other experimental set ups, its scope of application should be consistently expanded to include the investigation of plant uptake behavior of organic and emerging contaminants. Consequently, this study discusses the results from two applications of an optimized RHIZOtest procedure, on plant uptake of i) organic brominated flame retardants (four polybrominated diphenyl ethers (PBDEs) and four novel brominated flame retardants (NBFRs)) and ii) nanoplastics (in-house made Pd-enriched NPs). The main optimization consisted in using stainless steel over polyamides for the 30-µm mesh used to separated soil from roots, to avoid any possible adsorption of contaminants. In particular, uptake and translocation were investigated in tomato plants exposed to environmentally relevant soil-concentrations (i.e., 5 or 50 ng g<sup>-1</sup>dw for each PBDEs and NBFRs in soil, and 0.4,

and 4 mg NPs g<sup>-1</sup> dw for NPs in soil), achieved by spiking the tested contaminants into soil samples. Post-exposure, all roots and shoots replicates showed concentrations of the analyzed contaminants, demonstrating occurred uptake and translocation. For PBDEs and NBRs, roots and shoots concentrations ranged from 0.23 to 18.51 ng g<sup>-1</sup>dw and 0.09 to 6.85 ng g<sup>-1</sup>dw, respectively. For NPs, average concentrations ranged, between treatments, 0.09 – 1.24 mg NPs g<sup>-1</sup>dw for roots and 0.02 – 0.32 mg NPs g<sup>-1</sup>dw for shoots. From data quality assessment, no cross-contamination and negligible losses of spiked contaminants were assumed and good repeatability (i.e., low variability) of the experimental data was ensured. These results suggest profitable RHIZOtest potential future application on diverse contaminants and scenarios to refine the estimation of uptake parameters to be used in risk analysis.

### **3.08.P-Tu190 Uptake of contaminants of emerging concern and AMR into edible plants: a field study from Australia** *Minna Saaristo<sup>1</sup>, Kara Fry<sup>1</sup>, Erinn Richmond<sup>1</sup>, Laura Carter<sup>2</sup>, Mike Williams<sup>3</sup> and Mark P Taylor<sup>1</sup>, (1)Environment Protection Authority Victoria, Australia, (2)School of Geography, University of Leeds, United Kingdom, (3)Commonwealth Scientific & Industrial Research Organisation (CSIRO), Australia*

Irrigation of agricultural crops with recycled wastewater is becoming an increasingly important means of ensuring consistent access of water in Australia. Recycled wastewater is known to contain a vast array of chemical and biological contaminants, even after various treatment barriers. There is growing concern that these contaminants may also affect crops exposed to recycled wastewater irrigation. Accordingly, the aims of the project were to: 1) Detect and quantify a broad range of potential organic, inorganic and biological contaminants in two classes (A and B) of recycled wastewater in Victoria used for crop irrigation and their contribution to accumulation in soils and plant tissues, relative to reference sites; 2) Quantify contaminant concentrations in below (roots) and above (leaves, fruits) ground plant tissues to unravel uptake and distribution within plant tissues of a broad suite of contaminants under field conditions; 3) Measure indicators of plant condition (e.g., physiological traits) to assess whether recycled wastewater has an adverse impact on crop health and productivity. Plant, soil and water samples were collected from five farms using wastewater irrigation for edible crops (Class A) or pasture (Class C) and from two reference farms (i.e. not using recycled wastewater for irrigation). Total of 143 samples were collected, of which 41 were plants (roots, shoot floret), 41 soil, and 55 water samples. Broccoli, pasture, and lettuce were harvested along with adjacent soil and irrigation water. Our study shows that of the 1000 organic chemicals targeted, 6 were detected in water, 160 in soil and 100 in plant tissues. For example, 100 pesticides, 12 PPCPs, 30 PAHs, 25 substituted benzenes, 24 industrial chemicals, 15 phthalates, and 7 PFAS were detected. Of the nine AMR genes targeted, seven were measured in water, six in soil and two in broccoli tissues. Overall, exposure of pasture and edible crops to two different classes of recycled wastewater led to the uptake of a range of chemical and biological contaminants in edible crops. For instance, along with PPCPs and EDCs, other contaminants including PFAS, industrial chemicals, pesticides and AMR genes were detected. Despite this, our study shows no clear distinction between crops exposed to recycled wastewater and reference sites. Further analysis and assessment of contaminant residues in crops is underway to ensure that the use of recycled wastewater for irrigation is safe.

### **3.08.P-Tu191 Understanding the Role of Soil Properties in the Fate and Behaviour of Pharmaceuticals in Soil and Pore Water**

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Pharmaceuticals may enter agricultural environments directly from livestock excreta, or indirectly following irrigation with wastewater effluent or fertilisation with biosolids or manure. Once pharmaceuticals have been introduced to agricultural environments they may be taken up into crops, remain in the soil profile, degrade into metabolites or be leached from the system. The fate and behaviour of pharmaceuticals in agricultural environments has important implications for human and environmental health.

Whilst significant evidence on the uptake of pharmaceuticals into crops exists, our understanding of the effects of different soil properties on the fate, behaviour and uptake of pharmaceuticals is limited. This study aimed to address this by: 1) quantifying sorption behaviour of pharmaceuticals across a range of six different soil types with differing physical and chemical properties and 2) investigating the degradation of pharmaceuticals in both the soil and pore water of the same soils. The selected study pharmaceuticals were carbamazepine, lamotrigine, and sulfadimethoxine. Selected soils were characterised in terms of their pH, cation exchange capacity, organic carbon content and particle size distribution.

Sorption studies were conducted according to the Organisation for Economic Co-operation and Development (OECD) guideline 106 and were tested in the range 25-400µg/L. To understand pharmaceutical persistence, soil samples were separately spiked with the study pharmaceuticals at concentrations equivalent to 10mg/kg. The spiked soil samples were placed in a controlled temperature room at 21°C and held at 60% of the maximum water holding capacity. Soil and porewater samples were taken on days 0, 7, 14 and 40. Soil samples were then extracted using methanol analysed by HPLC-MS.

Adsorption-desorption distribution coefficients (K<sub>d</sub>) across the tested soils ranged from 1.7-11.0 L/Kg for carbamazepine, 5.6-40.1 L/Kg for sulfadimethoxine and 5.0-15.7 L/Kg for lamotrigine. Sorption isotherms were linear across the concentration range tested. The greatest K<sub>d</sub> values were observed in the soils with the highest organic carbon (%C) contents and highest cation exchange capacities. The significance of pH and particle size were compound dependent.

This study provides valuable insight into the differing fate and behaviour of pharmaceuticals across a range of soil types, and will contextualise future studies of plant uptake of pharmaceuticals.

### 3.08.P-Tu192 Sorption, Persistence, and Plant Growth Inhibitory Activity of S-Absciscic Acid in Soils

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Natural plant growth regulators (PGRs), primarily represented by phytohormones, are gaining increasing attention in modern agriculture as eco-friendly agrochemicals to improve the yield and quality of crops under increasingly severe environmental conditions. However, the fate of these compounds once they enter the subsurface soil environment has received very little consideration. Information regarding the behavior of natural PGRs in soils is necessary to anticipate their residual activity and associated non-target effects. S-absciscic acid (S-ABA) is a plant growth inhibitory phytohormone currently authorized for several uses in agriculture at a maximum application rate of 2 kg/ha. Little is known, however, about the sorption and dissipation processes S-ABA may undergo in different soil types and even less about how its activity in soils may differ from that observed under soilless conditions or upon its direct application to plant tissues. In this work, we characterized the sorption and dissipation of S-ABA in three soils, two alkaline and one acid, and evaluated how the soils altered the plant growth inhibitory activity exhibited by S-ABA under soilless (Petri dish) conditions. S-ABA displayed low sorption coefficients in all soils ( $K_d < 0.12$  l/kg), and dissipation times ( $DT_{50}$ ) ranged between 0.7 and 8.4 days. Notably, S-ABA displayed greater sorption and persistence in acid soil compared to alkaline soils, and both sorption and dissipation decreased with the initial concentration of S-ABA, implying increased bioavailability at higher soil concentrations. In the presence of the soils, the inhibitory concentrations ( $IC_{50}$ ) of S-ABA for the germination and seedling growth of *Eruca sativa* became 3 to 125 times greater than those measured under soilless (Petri dish) conditions. Biodegradation, rather than sorption, was identified as the primary process weakening the activity of S-ABA in the soils. Despite the reduction in the activity of S-ABA provoked by the presence of the soils, the phytohormone still expressed its inhibitory activity at relatively low soil concentrations (0.3-20 mg/kg), which could be important in assessing its residual activity and non-target effects derived from its use as an agrochemical. Acknowledgment: Funded by the Spanish Ministry of Science and Innovation through grants PID2020-112563-RB-I00 and PRE2021-100664.

### 3.08.P-Tu193 Multiscreening of pesticides in plant biomass for detection of groundwater contamination

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Background: Pesticides are the organic molecules designed to protect and enhance agricultural production. Although the application of these substances is targeted to agricultural areas, trace amounts can be found in all environmental compartments due to the persistence and mobility of parent substances and degradation products. Pesticides are also a major contributor to groundwater pollution. The aim of this study is to use vegetation to detect and monitor groundwater contamination using phytoscreening, and to use this approach also to protect water resources through protection zones.

Methods: For the first part of the study, a hydroponic experiment was conducted with 16 pesticides and black poplar seedlings. Simultaneously, an LC/HRMS method for the determination of pesticides and relevant metabolites was also developed and optimized involving sample treatment using QuEChERS with ChloroFiltr and SPE approaches. Due to the different stability, structural and physicochemical properties of the compounds, no single preparation method could be used. The obtained sample treatment methods allow a reduction of matrix effects by up to 60%. Recovery and reproducibility of the methods were within the specified limits. The hydroponic medium and plant biomass (roots, trunks and leaves) were analyzed by target and non-target analysis for determination of the parent compounds, metabolites and changes in secondary plant metabolism.

Results and conclusion: Azole fungicides were one of the monitored groups. The target analysis showed the presence of azole fungicides in all parts of the plants. In this case, transport of the substance from the roots to the leaves was observed. This trend was more favorable for tebuconazole due to its vapor tension and Henry's constant. Furthermore, the non-target analysis of hydroponic medium and plant material was performed. The analysis of hydroponic medium showed the presence of hydroxylated transformation products of tebuconazole and degradation products of propiconazole. Similar products were also found in extracts of plant parts. In addition, conjugation of these compounds with glucose occurred in the plant biomass. Their relative increase from roots to leaves was observed. In the context of azole fungicides, the fate of 1,2,4-triazole in plant tissue was investigated. It was observed that during transport from the root to the leaf part, changes in the representation of triazole lactic and triazole acetic acids occur.

### 3.08.P-Tu194 Small-molecule Fingerprinting for Contaminant Screening in Reclaimed Water and Non-target Analysis of the Irrigated Rice

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Reusing reclaimed water for rice irrigation is a potential resolution to mitigate water scarcity. However, reclaimed and conventional irrigation water differ in chemical composition, and the effects of reclaimed water irrigation on crops are not fully clarified. Aiming to elucidate the contaminants in reclaimed water and the effects on irrigated rice, this study fingerprinted small molecules in water and rice.

Reclaimed (n =10) and river (n =10) water used to irrigate rice paddy were collected from March to November 2023. Water samples were prepared by filtration and solid-phase extraction. Thirty-five whole rice plants were sampled for each irrigation group (reclaimed or river water). The homogenized white rice samples were processed through solvent extraction and cartridge cleanup. The samples were analyzed using liquid chromatography–high-resolution mass spectrometry.

The molecular features in water samples were searched in the database, which recorded around 2800 contaminants. The features in the rice with high variabilities (relative standard deviation, RSD >30%) were filtered. Multivariate analysis models were constructed to compare the distribution of rice samples between groups. The selection of candidate markers of reclaimed water irrigation was conducted based on the variable importance in projection (VIP) scores in the partial least squares discriminant analysis (PLS-DA) model.

Thirty-six and 14 contaminants with detection rates above 50% were found in the reclaimed and river water, respectively. Twenty-five contaminants were only detected in the reclaimed water, probably resulting from the compromised removal efficiency of wastewater treatments. Although only three contaminants were found solely in the river water, their control is challenging given the diverse sources. As for rice, nearly 60% of the molecular features exhibited low RSDs (<30%) in the pooled samples, indicating high analytical reproducibility. The PLS-DA model constructed using 2240 features significantly discriminated between rice irrigated with two waters ( $R^2 = 0.751$ ,  $Q^2 = 0.661$ ). Ninety-five features were selected as candidate markers (VIP >2).

This study successfully applied contaminant screening and non-target analysis to illustrating the small-molecule distribution in water and rice. Different small-molecule fingerprints in two waters and the rice of two irrigation groups were evident. Further studies will include a whole-year rice investigation and marker identification.

### **3.08.P-Tu195 Multitarget and suspect-screening of antimicrobials in vegetables samples: uptake experiments and identification of transformation products**

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Antimicrobial (AM) contamination in vegetables and the formation of unknown transformation products (TPs) have raised concern about the emergence and dissemination of resistant-bacteria. Within this context, an accurate analytical method to perform a multitarget analysis of five sulfonamides, four tetracyclines, four macrolides, nine fluoroquinolones and quinolones, one imidazole and one nitroimidazole, one triazole, one diaminopyridine, one derivative of *Penicillium stoloniferum* (DP) and three antifungals in several vegetable samples (lettuce, onion, tomato, and carrot) using liquid-chromatography coupled to a low-resolution triple quadrupole mass spectrometer (UHPLC-MS/MS) was developed. Moreover, the method was extended in order to monitor the potential TPs by means of UHPLC coupled to a high-resolution mass spectrometer (HRMS) using an UHPLC-q-Orbitrap. Both analysis techniques were instrumentally compared in terms of limits of quantification and matrix effect at the detection. The method was applied to determine the presence of AMs in different case studies. On the one hand, the evaluation of AMs in organic and non-organic vegetable samples (lettuce, onion, tomato and carrot) acquired in supermarkets from the Basque Country (Spain) shown the presence of sulfadiazine and mycophenolic acid in carrots regardless of the agriculture type. On the other hand, the potential transference capacity of some AMs (trimethoprim, sulfamethazine, enrofloxacin and chlortetracycline) from soils to vegetables, using lettuce as a model, was evaluated through controlled uptake experiments. In this case, each of the AMs previously spiked in the soil were also found in the exposed lettuces. In both case studies, specific TPs of the target analytes were identified. For instance, (4E)-6-(4,6-dihydroxy-7-methyl-3-oxo-1,3-dihydro-2-benzofuran-5-yl)-4-methylhex-4-enoic acid, derived from the demethylation of mycophenolic acid, was tentatively identified and found in carrots from organic and non-organic agriculture, whereas 1-cyclopropyl-7-[(1Z)-2-(ethylamino)ethenyl]amino}-6-fluoro-4-oxoquinoline-3-carboxylic acid, derived from enrofloxacin, was detected in the exposed lettuce samples.

### **3.08.P-Tu196 The influence of Bio-based fertilizers (BBFs) on the degradation of anionic pharmaceuticals**

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Pharmaceutical residues have been increasingly identified in agricultural soil due to anthropic activities. Bio-based fertilizers

(BBFs), as sustainable alternatives to chemical fertilizers, can help close nutrient loops, while affecting the degradation of pharmaceuticals in soil. To comprehensively understand this influence, degradation experiments were performed in which four pharmaceuticals (naproxen, ketoprofen, diclofenac and ibuprofen) were spiked in soils previously amended with a plant-based or an animal-based BBF. The data generated from the experiments were compared to those from a control without BBF addition and used to develop a degradation model for the first time that takes into account the distribution of pharmaceuticals in soil over the bioavailable fraction (BFS) consisting of the dissolved and rapidly desorbing fractions, and the strongly adsorbed fraction (SDS). The dynamics of pharmaceutical degradation based on the fate of the total extractable fractions (TES) encompassing the BFS plus the SDS fraction generally followed a single first-order model. After the addition of BBFs to the soil, the ratio of the bioavailable to the total extractable fractions i.e. SDS to TES increased, indicating an enhanced, strong sorption capacity for each pharmaceutical compound. This shift implies that a larger proportion of these compounds had a higher tendency to be adsorbed by the amended soil, reducing the bioavailable fraction for degradation. Simultaneously, we observed a decrease in the overall degradation rates of all fractions, including the BFS fraction for each pharmaceutical upon BBF amendment. This study presents a valuable model for elucidating the fate of pharmaceutical compounds in soil and the effects of BBFs on their persistence, taking into account their distinct chemical properties and the intricate interplay of each fraction in both soil and amended soil. Moreover, these methods hold considerable potential for assessing the consequences of wastewater irrigation on pharmaceutical degradation and how BBFs influence the uptake of pharmaceuticals by plants in soil.

### **3.08.P-Tu197 Occurrence of Microplastics in Soils Irrigated With Reclaimed Water**

*Jose Javier Flores Morales, Laura Cortés Corrales, Amadeo Rodríguez Fernández-Alba and Maria Jesus Martinez Bueno, Chemistry and Physics Department, University of Almería, Spain*

Suggesting the use of reclaimed water for agricultural irrigation offers a practical solution to combat water scarcity in the coastal regions of Mediterranean countries. To date, reclaimed water for crop irrigation has been poorly evaluated in terms of microplastics (MPs) load. Extended exposure to advanced treatment processes results in the gradual decomposition of plastic materials into numerous smaller plastic particles known as MPs. Those particles are accumulated in large quantities in agricultural soils irrigated with reclaimed water, posing a potential threat to terrestrial ecosystems. Despite the intensive use of plastics in agriculture, little is known about the presence of MPs in the agro-environment. Long-term plastic pollution may even lead to a further reduction of agricultural production. However, the extent to which terrestrial ecosystems, especially agri-food systems, are affected is still largely unknown. Thus, the main objective of this work was to determine the presence and/or accumulation of MPs in agricultural soils irrigated with reclaimed water in protected crops (greenhouses).

For the analysis of MPs in the reclaimed water samples, 25 µm stainless steel filters (volume 30 L) were used. The filters were subjected to an ultrasonic bath and the organic solvent filtered through a 0.45 µm nitrocellulose filter. Soil samples were sieved on stacked stainless steel sieves with mesh sizes of 1 mm, 300 µm, 100 µm and 25 µm. The fractions were then dehydrated in an oven at 60 °C for 24 hours. The MPs were separated from the soil matrix by density flotation using a saline solution. Finally, a stereomicroscope in combination with a micro-FTIR was the instrumentation used to identify the number of MPs and classify them in terms of morphology, color, and type. Preliminary results showed that reclaimed water is a source of MPs. Fibers and fragments were the most abundant MPs in the reclaimed water samples analysed. A total of 5 different types of polymers and only 1 non-plastic material (cellulose) were identified. The polymers detected by FTIR analysis were PTFE, PET, PP, PE and PVC. The work supports the reuse of water for agricultural irrigation. Nevertheless, reclaimed water constitutes a source of MPs, and its presence should be evaluated in future studies.

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### **3.08.P-Tu198 RISK ASSESSMENT OF ORGANIC MICROCONTAMINANTS AND MICROPLASTICS IN PROTECTED CROPS IRRIGATED WITH RECLAIMED WATER**

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Reclaimed water contributes to mitigating water stress and offers a sustainable alternative for farmers in areas which suffer from greater water scarcity. Some scientific studies have documented the presence of organic microcontaminants, such as pharmaceuticals or pesticide residues, in reclaimed water. But, new contaminants, plastic waste, are posing a new challenge to the scientific community. To date, reclaimed water for crop irrigation have been poorly evaluated yet in terms of microplastics (MPs) load. In recent years, studies have shown that the use of reclaimed water for crop irrigation can be an important pathway for the introduction of organic pollutants into agricultural production and their subsequent entry into the food chain; this could pose a risk to health and the environment. Thus, due to the increasing presence of these pollutants in the environment and their potential negative effects on ecosystems and human health, an assessment of the possible risks derived from the use of reclaimed water for agricultural irrigation of protected horticultural crops in greenhouses is relevant.

Almería is the main production area of horticultural products for Spain and Europe. According to data published in a recent report by the Junta de Andalucía, 48% of Almeria's farmers use reclaimed water as their sole source of supply. The goal of the work was to provide scientific-technical knowledge regarding the impact on the water-soil-plant continuum of using reclaimed

water for commercial crops irrigated over a long period, as well as the human risks associated with consuming the vegetables produced.

Reclaimed water is a source of MPs and organic microcontaminants. PTFE, PET, PP or PE were the most frequently detected polymers in reclaimed water. The accumulation rate of the organic contaminants detected in the edible parts of the vegetables permanently irrigated with reclaimed water was very low (~1%), whereas it was 33% in the soils. Thus, the results revealed that consuming fruits harvested from plants irrigated for a long period with reclaimed water does not represent a risk to human health. Nonetheless, the study highlights the importance of carrying out a long-term control strategy on agricultural soil that is permanently irrigated with reclaimed water to avoid high accumulation rates among certain organic microcontaminants that could migrate over further crop seasons.

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### **3.08.P-Tu199 Assessment of a multi-test protocol investigating fate and ecological impacts on soil environment due to potential contaminants in fertilizers**

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A multi-test experimental protocol was developed to gather empirical data for risks analysis, investigating the exposure of the human food chain to crops grown in agricultural soil treated with fertilizing products that may contain contaminants. To ensure repeatability and reliability of generated data, the proposed activities within the protocol are planned to be performed accordingly to internationally validated standards. In particular, both chemical availability and bioavailability of contaminants were assessed, by leaching tests with different media simulating interactions between soil, chemicals and roots exudates, and by the performance of the so called RHIZOtest (ISO 16198), to experimentally investigate their plant uptake. This latter was proposed due to its acknowledged benefits over hydroponic- and non-standard soil-based tests.

The experimental activity addressed a single solid fertilizer product, composed of green compost, chicken manure, urea, triple superphosphate and potassium chloride, specifically manufactured by the authors in accordance with the requirements for organo-mineral solid fertilizers laid down in Regulation 2019/1009/EU.

Together with a sample of fertilizer products alone, one sample of standard soil alone, each soil-fertilizer sample previously prepared underwent leaching (extraction) test using 3 different leaching media to simulate rhizospheric processes (H<sub>2</sub>O demi, a 0.01 M solution of CaCl<sub>2</sub> and a 0.015 M solution of oxalic acid) with a L/S ratio of 10:1 (EN ISO 18772 and EN ISO 17402). Each leaching test was performed in triplicate and analyzed to determine its chemical composition. Simultaneously, each soil-fertilizer test sample, including the untreated soil control, were subjected to plant uptake tests following the so called RHIZOtest configuration, standardized as per ISO 16198.

Finally, the investigated fertilizer products were subjected to ecotoxicological characterization using representative organisms from both terrestrial and aquatic environments, through methods align with those recognized by the scientific community for waste hazard classification (HP 14 – Ecotoxicity) and the regulation for product classification and labeling and the analysis methods provided therein 440/2008/EC.

### **3.08.P-Tu200 Evaluation of the potential toxicity of swine wastewater treated with recycled oyster shell and pumice in soil environments**

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Despite the abundant nutrients that could be reutilized in swine wastewater, excessive metals, such as copper and zinc, and inadequate wastewater management can engender ecological impacts to the aquatic and terrestrial ecosystems. Herein, this study aims to reuse oyster shell and pumice waste as filter medium for swine wastewater treatment, intercepting nutrients for agricultural application. To assess the potential toxicity of treated medium in soil environments, this study employed the soil nematode (*Caenorhabditis elegans*) and Chinese cabbage (*Brassica rapa chinensis*) assays, including growth assays and seed germination assay. In addition, the field test of the treated medium was conducted in farmland with corns and cucumbers. Our findings showed the nutrients and metals were largely removed from the swine wastewater treated with oyster shell and pumice. For *C. elegans* assays, the growth and reproduction were not adversely affected by treated medium, whereas the non-treated air-dried sludge of wastewater completely inhibited the growth and reproduction of *C. elegans*. For Chinese cabbage assays, the treated medium (10%–40%) did not significantly inhibit germination and growth of Chinese cabbage, whereas the non-treated air-dried sludge of wastewater would be harmful to Chinese cabbage growth. Furthermore, the treated medium (20%–40%) could further promote growth of Chinese cabbage. In farmland, the treated medium (30% and 50%) slightly increased the corn growth. In addition, the total cucumber harvest was increased by 224% in treated medium group (50%). In conclusion, recycling oyster shells and pumice for swine wastewater effectively reduces contamination, and the treated medium has no significant adverse effects to soil environment and agricultural use, supporting its reuse suitability and enhancing the value of oyster shells, pumice, and swine wastewater while minimizing environmental impact.

### 3.08.P-Tu201 Health Impact of Vegetable Consumption from Urban Gardens in Andalusia, Spain

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Urban food production plays a crucial role in mitigating climate change by reducing the need for food transportation, packaging and energy consumption. Plants acquire essential elements from the air and soil. However, human activities contribute to increased potentially toxic elements (PTEs) concentration in urban areas. Therefore, it is imperative to monitor the quality of food, as plants serve as a primary pathway for PTEs to enter the food chain. Urban soils display notable diversity in their physicochemical characteristics, indicating a broad spectrum of contamination levels. Various vegetable species were gathered from urban gardens in Seville, Cordoba, and Huelva (Southern Spain), as well as from two small towns in a mining region (Riotinto), along with topsoil samples near the plants. The levels of potentially toxic elements (As, B, Ba, Cd, Co, Cr, Cu, Mo, Ni, Pb, and Zn) were assessed in the edible parts of the plants and the soil. Additionally, the same species obtained from the local market in Seville and a peri-urban area (a domestic garden in a rural region) were analyzed. The relationship between plant/soil pollution was examined, and the potential risks to human health were evaluated using various parameters. The soils in urban gardens from the mining area exhibited higher contamination levels compared to the others, and generally, the soils in the city displayed higher values of potentially toxic elements than those in the peri-urban area. Leafy species generally had higher mean concentrations of almost all potentially toxic elements compared to fruiting and bulbous species. Arsenic, Cd, and Pb concentrations were below the health-based guidance values in all vegetables, except for Cd in one sample from the peri-urban area. Overall, the vegetables from urban gardens in the city did not exceed the market concentrations for most of the studied elements, except for Pb in onion, lettuce, and chard. The Hazard Quotient (HQ) values for all elements in plant species from the studied gardens were below one, as were the Hazard Index (HI) values, indicating that the consumption of these vegetables can be considered safe and poses no risk to human health. Furthermore, the cancer risk values for As were below the established limits in all vegetables from the studied urban gardens, including the mining area, suggesting that urban agriculture in this region is safe, even in soils contaminated with high levels of As and Pb.

### 3.08.P-Tu202 Assessing the Efficacy of Reactive Barriers in Mitigating Emerging Contaminants: Laboratory Insights into Aquifer Recharge with Treated Wastewater

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Water scarcity is a pressing global concern exacerbated by population increase and the adverse impacts of climate change. Declining water resources require immediate attention, particularly in the context of depleting aquifer levels. Managed aquifers recharge has emerged as a critical strategy to sustain water availability. However, the use of treated water from wastewater treatment plants for aquifer recharge requires additional treatments due to the presence of emerging contaminants, such as pharmaceuticals and personal care products.

This study focuses on enhancing the efficiency of aquifer recharge by employing reactive barriers, which serve as a final treatment step for water infiltrating aquifers. To evaluate the effectiveness of these barriers, laboratory experiments were conducted using column setups that mimic reactive barriers. Over a three-month period, treated wastewater from a sewage treatment plant was injected through a sand column (representing conventional aquifer conditions) and a barrier column composed of a mixture of natural materials, including compost, sand, and wood.

The results reveal that certain molecules, notably venlafaxine and metoprolol, exhibit significant retention in the barrier columns, highlighting the efficacy of the barrier. Conversely, some molecules, such as irbesartan, show partial retention in both media. This study comprehensively presents the spectrum of molecules tracked during the experiment, along with associated sorption and degradation models. The discussion focuses on the effectiveness of the barrier in dealing with the fate of emerging contaminants, highlighting the importance of such strategies in water resource management.

### 3.08.P-Tu203 A Higher Sensitivity of HMA Genes and Better Photosynthetic Performance Led to Improved Cd Phytoextraction by *Brassica napus* under Future Climate

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Changing climatic conditions undoubtedly influence heavy metal behavior and fate in the environment and thus influence the plant-heavy metal interaction. This study aimed to investigate the underlying physiological, biochemical, and molecular mechanisms responsible for *Brassica napus*'s potential to remediate Cd-contaminated soil under current climate (CC, 400 ppm of CO<sub>2</sub> and 21/14 °C) and future climate (FC, 800 ppm of CO<sub>2</sub> and 25/18 °C) conditions. *B. napus* exhibited good tolerance to low Cd treatments (Cd-1, Cd-10, i.e., 1, 10 mg kg<sup>-1</sup>) under both climates without visible phytotoxicity symptoms. The tolerance index (TI) of shoots sharply decreased by 47% and 68% ( $p < 0.05$ ), respectively, in Cd-50 and Cd-100 treated shoots under CC, but to a lesser extent (-26% and -53%,  $p < 0.05$ ) under FC. This agreed with increased photosynthetic apparatus performance under FC, primarily due to the higher amount of active reaction centers (RCs) of photosystem II (PSII). It was indicated by a higher density of active RCs on PSII antenna chlorophyll *a* basis (RC/ABS) and per excited cross section (RC/CSm), lower increases in the rate of RCs closure (dV/dt)<sub>o</sub>, and less dissipated excitation energy (DIo/RC, φDo), especially under Cd-100. Calvin Benson cycle (CBC)-related enzyme activity also improved under FC with 2.2-fold and 2.4-

fold ( $p < 0.05$ ) increases in Rubisco and triosephosphate isomerase (TPI) under Cd-50 and Cd-100, respectively. As a result, a 2.2-fold and 2.3-fold ( $p < 0.05$ ) increase in photosynthetic rate ( $P_r$ ) resulted in a 2.3-fold and 2.4-fold ( $p < 0.05$ ) increase in the shoot dry weight of Cd-50 and Cd-100 treated rapeseed, respectively. This also led to a decrease (26%,  $p < 0.05$ ) in shoot Cd concentration under both high Cd treatments with a slight reduction in bioconcentration factor (BCF). Translocation factor (TF) decreased (on average 42%,  $p < 0.05$ ) by high Cd treatments under both climates. However, under Cd-100, FC increased TF by 1.7-fold ( $p < 0.05$ ) compared to CC, which was explained by significant increases in HMA genes, especially *BnaHMA4a* and *BnaHMA4c*, expression. Finally, the total uptake (TU) index of Cd increased under FC by 65% and 76% ( $p < 0.05$ ) under Cd-50 and Cd-100. This led to a shorter hypothetical remediation time for reaching the Cd pollution limit by 35 ( $p > 0.05$ ) and 61 ( $p < 0.05$ ) years, respectively, compared to CC.

Keywords: *B. napus*, Phytoextraction potential, HMA genes, Photosynthetic performance, CBC-related enzymes, Future climate

### **3.09.A Complex Mixtures of Chemicals in the Environment and the Human and Eco-Exposome – Next Generation Monitoring (NGM), Toxicity Driver and Source Tracking to Meet Regulatory Needs**

#### **3.09.A.T-01 Feature Tracking in NTS: Staying In Touch With The Unknown**

*Geert Corné Franken and Eelco Nicolaas Pieke, Het Waterlaboratorium, Netherlands*

The application of non-target screening (NTS) for water monitoring is essential for a comprehensive understanding and management of environmental contaminants. Traditional NTS methods excel in detecting unknown substances, but often lack a systematic approach for naming and tracking these substances, which is essential for detailed analysis and comparison. Therefore we introduce a new methodology for feature tracking in high-resolution LC-MS NTS, focused on the principle of "Leave No Feature Unnamed." Key to this method is the understanding of the important role of metadata in prioritizing, annotating, and selecting features accurately and based on evidence. This methodology also contributes to clearer reporting of results, allowing for adjustments in retention time (rt) and mass-to-charge ratio (mz) prior to final reporting. An important part in this methodology is the Feature Tracking Database (FTDB), where systematically both analytical data and extensive metadata of features is stored, facilitating their detailed tracking.

To assess this methodology, three years of routine NTS data from two critical monitoring stations along a Dutch river, a significant source of drinking water, and a related drinking water production facility was processed. The application of the workflow allowed for the systematic organization and storage of features found at these locations in the FTDB. This enabled the prioritization of key features appearing in both the river and drinking water samples, showcasing the potential of the methodology for environmental monitoring.

As an evolving concept, this methodology is open for collaboration and the exchange of expertise for further development. This feature tracking methodology aligns with significant initiatives such as the European Commission's Green Deal and the Partnership for the Risk Assessment of Chemicals (PARC), marking a step towards integrating NTS into regulatory frameworks and advancing towards a sustainable, pollution-free environment.

#### **3.09.A.T-02 Cross-laboratory Non-Target Screening in an International River Catchment – the Case for Monitoring the Rhine River and its Tributaries in Real-Time**

*Martin Loos, enviBee, Switzerland*

The rise of non-target screening (NTS) has enabled a concise monitoring of long-term micropollutant trends at various sampling points in a broad range of surface waters. While the analytical methodology is steadily improving and its results are becoming gradually accepted among regulatory bodies, a framework to harmonize and compare NTS data in large transnational catchments at short timescales has been lacking to date. This shortcoming has impeded the fast detection and tracking of pollutant trends and their sources of emissions across boundaries and has not allowed to bundle all available information and forces from existing laboratories to prioritize and communicate nontarget masses for identification efforts.

We therefore present a harmonized and vendor-independent approach to compare LC-HRMS data across monitoring sites, combined with a real-time data mining and synchronization routine on a common server. While data from each sampling point in a river network is measured and profiled in a user-friendly workflow by one of several participating laboratories, the synchronization routine automatically screens for any updated data in the background. The new data is then aligned, its cross-laboratory comparability estimated and the annotated LC-HRMS masses grouped and stored in a joint database for various query purposes. The database can then be searched with a convenient user interface on the same server. Therein, laboratories can also dynamically merge their sets of known compounds, as well as exchange information on non-targets and trends of interest on a joint platform. The framework has been thoroughly tested and streamlined by five participating laboratories in the catchment of the river Rhine, paving its way for daily international routine usage.

The presented implementation makes a strong case for pushing NTS past retrospective and locally restricted screening, and towards a real-time and distributed next generation monitoring of pollutant dynamics. We underline its feasibility with several



use cases which have led to the successful prioritization and identification of non-target compounds of catchment-wide concern for the protection of an international water body.

### **3.09.A.T-03 Development and Application of Non-Target and Suspect Screening for Environmental Chemical Regulatory Purposes in the United Kingdom**

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The diversity of chemicals entering the environment present a challenge for effective regulation of chemicals, as few data are available for a large proportion of these chemicals. A fundamental obstacle to assessing the environmental risk of chemicals is that traditional targeted monitoring covers a small proportion of the chemicals that are produced or used. Selecting chemicals to focus monitoring, research, and regulatory efforts on requires prioritisation of those that are expected to pose the greatest environmental risk. Monitoring approaches using high-resolution mass spectrometry (HRMS) followed by non-target/suspect screening workflows can contribute towards this process by providing preliminary information about exposure to a much broader range of chemicals, permitting a more holistic assessment of environmental quality. These approaches can also contribute towards identification of chemical mixtures responsible for environmental incidents and can provide information about chemical effects on organisms when implemented in combination with biological information, such as from effect-based methods, through effects-directed analysis. The Environment Agency in England is working with other UK and international organisations to develop and apply non-target and suspect screening approaches to enhance chemical management strategies in the UK. These techniques are being applied in various workstreams, and some of these will be showcased in this presentation. These include the development of in-house methods for analysing water, sediment, soil and biota samples. Suspect screening is also being applied to monitor chemicals of emerging concern in Eurasian otters (*Lutra lutra*) as part of the H4 Otter Project, in passive samplers, and in wastewater influent and outputs as part of the Chemicals Investigation Programme (CIP). A UK cross-government Working Group for Non-Target and Suspect Screening has also been initiated to provide a forum for exchanging knowledge using a co-ordinated approach and to set priorities that are relevant to the UK environment. We will explain how the use of non-target and suspect screening approaches offers multiple benefits and how limitations and challenges can be managed, leading to simplification of monitoring protocols and enabling complex data evaluation, storage and sharing.

### **3.09.A.T-04 The innovative pull-down approach as an efficient tool for the identification of endocrine-disrupting compounds in environmental mixtures**

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Endocrine-disrupting compounds commonly occur in environmental mixtures as it has been widely documented by various bioassays. Nevertheless, even though several methods, like effect-directed analysis, are used for effect driver identification, the drivers of endocrine-disrupting effects often remain unknown. Recent studies document that complex environmental mixtures associated with discharges from wastewater treatment plants can contain compounds disrupting thyroid hormone transport facilitated by transthyretin (TTR protein), which is crucial for proper neuro/development and metabolism in vertebrates. We have developed and optimized a novel method called a pull-down assay to identify them in a water matrix. This method is based on separating TTR ligands from a sample by interaction with designed, expressed, and purified TTR protein. Immobilized TTR protein (through its Histidine tag binding to magnetic particles) with its ligands is separated from the rest of the sample. Protein is degraded, and ligands are identified using non-target HPLC-HRMS analysis and confirmed using TTR bioassay.

Extracts from water samples were used in pull-down assay to trial the real-world use of the TTR pull-down workflow. Non-target HPLC-HRMS analysis identified twenty-two compounds, of which thirteen were verified by their standards using TTR bioassay, including diclofenac and its metabolites, linear alkyl benzene sulfonates (LAS), lupulone, citalopram, and telmisartan. Among these compounds, we identified several pharmaceuticals and their metabolites that showed even higher affinity to TTR than the parent compound. An interesting identified group was LAS, which accounted for a major part (5.5% - 39%) of the TTR-binding inhibition effect of the field samples, which we could explain with known and newly identified ligands. These findings emphasize the need to investigate the possible endocrine-disrupting effects of commonly used chemicals and their metabolites released into the environment. We document that pull-down assay can be a valuable versatile method for identifying ligands from different samples through almost any expressible protein for various purposes.

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### 3.09.A.T-05 Chemicals with Evidence for Presence in Humans and in Food Packaging

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Over 12,000 food contact chemicals (FCCs) may be intentionally used during the manufacture of food packaging and other food contact articles, and more than 1800 FCCs have been shown to migrate from food contact materials (FCMs) into the packaged foods or food simulants. Collectively we refer to these chemicals as “universe of known FCCs”. However, it has not been systematically assessed which of these FCCs have been detected in humans, although such an assessment is crucial to understand how FCMs contribute to human exposure to FCCs, encompassing potentially harmful chemicals and mixtures. Therefore, in a stepwise approach, we compared the known FCCs to metabolome/exposome databases and human biomonitoring programs. Then, we prioritized a set of FCCs without any evidence for presence in humans and performed a targeted systematic evidence mapping.

Twenty-five percent of the FCCs have varying types of evidence for presence in humans. In step 1, the majority of FCCs have been identified in metabolome/exposome databases. A subset of 194 FCCs has been monitored and detected in biomonitoring programs, while 70 FCCs have been monitored but never detected. Phthalates and alternative plasticizers and metals have been detected most frequently in humans and in FCMs, followed by volatile organic compounds, phenolic compounds, and per- and polyfluoroalkyl substances. Dioxin-like compounds, pesticides, and flame retardants have also been detected in humans, but they have been less frequently found in FCMs. Reasons could be contaminations or the illicit use of these chemicals during manufacture, as many of these FCCs are not listed for intentional use. Step 2 showed that antioxidants have a high level of evidence for presence in FCMs, but there is only limited information on their presence in humans. Whether this is due to data gaps or degradation of the FCCs will be a question for follow-up work. On the other hand, bisphenol A-based derivatives (like BADGE), commonly found in can coatings and known to migrate into foods, have a high level of evidence for presence in humans.

This study provides a systematic overview of FCCs with links to human exposure. With over 3500 FCCs, the FCCchumon database will be made accessible as a user-friendly, interactive and free dashboard. These data can also inform further research by indicating knowledge gaps and inform evidence-based decision-making, for example, by highlighting hazardous FCCs commonly detected in humans.

### 3.09.B Complex Mixtures of Chemicals in the Environment and the Human and Eco-Exposome – Next Generation Monitoring (NGM), Toxicity Driver and Source Tracking to Meet Regulatory Needs

#### 3.09.B.T-01 Advanced High- Throughput Effect-Directed Analysis (HT-EDA) Workflow for the Identification of Androgenic Compounds

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Effect-directed analysis (EDA) is a promising tool for understanding environmental sample mixtures and unravelling toxicity drivers, yet its routine use is hindered by time and labour intensity. However, advances in high-throughput (HT) fractionation systems and toxicity assessment have improved EDA's efficiency. Still, current HT-EDA approaches face some challenges mainly due to constrained chemical analysis conditions and the non-target screening bottleneck. In this study we have addressed these issues by exploring APCI alongside ESI for an expanded chemical space coverage, while mitigating increased feature detection with computational prioritization tools to focus on relevant compounds linked to specific toxicity endpoints. A hospital effluent sample with high androgenic activity was analyzed, employing the Fractionator™ system for microfractionation followed by androgenic activity biotesting by AR-CALUX. The chemical analysis, using an Orbitrap Exploris 480 with ESI and APCI ionization, was focused on both the original sample and detected toxic fractions. The non-targeted analysis strategy involved MZmine data processing, prioritization of potential androgens using the MLin vitroTox tool, and subsequent structure elucidation using SIRIUS and compound annotation using Massbank and endpoint specific suspect-lists. The AR-CALUX bioassay revealed up to 8 toxic fractions in the sample, guiding the focused chemical analysis on this retention time window. A two-level comparison of ESI and APCI ionization modes indicated a higher feature detection with ESI, but after prioritization with MLin vitroTox, the gap narrowed, and 13 potential androgenic features unique to APCI were detected, aligning with the decision to use ionization source to extend the detectable chemical space. The non-target results showed 60 prioritized features, with notable overlap between these features and toxic fractions. The in-house database match suggests the presence of compounds like 4-Androstene-3,17-dione, Testosterone, or 11-Ketotestosterone, emphasizing the utility of MLin vitroTox in focusing efforts on relevant compounds for further identification. The proposed effectively aligns with the requirements of HT-EDA, specifically addressing the need to expand the detectable chemical space and incorporating a non-target screening approach focused on endpoint-specific relevant candidates.

### 3.09.B.T-02 Effect-Directed Analysis in Human Serum Samples - the Challenges of Dealing with Endogenous Molecules

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Effect-directed analysis (EDA) is an innovative and advanced approach designed to pinpoint and evaluate potential risks associated with complex mixtures of chemicals. EDA integrates state-of-the-art analytical techniques with *in vitro* bioassay testing, which allow the identification of specific components responsible for observed toxic effects. Generally, EDA involves assessing the biological activity of the original extract, followed by its fractionation and subsequent biological and chemical analysis. The question is: are we missing important active chemicals in the analysis just because they coelute with endogenous molecules that appear at higher intensities in the mass spectra? The data processing workflow used for chemical identification is essential to elucidate which chemicals may trigger an effect on the bioassay. In this study, a high-throughput EDA approach was utilized to evaluate the capacity of chemical mixtures found in human serum samples to compete for the binding to thyroid hormone (TH) serum protein—an essential molecular initiating event linked to TH system disruption. Different data acquisition, annotation, mass spectra (MS) alignment and evaluation strategies are used to assess the chemical composition of the active fractions.

Extracts obtained from pooled human umbilical cord serum and various adult serum samples were subjected to high throughput EDA. Briefly, serum extracts and their matching procedural blanks were fractionated by high performance liquid chromatography (HPLC) coupled to a fraction collector (FractioMate™). The activity of the fractions was assessed using the a TTR-binding assay. Non-targeted chemical profiling of the extracts was performed using the same chromatographic conditions coupled to a qTOF mass spectrometer. Data Dependent Acquisition (DDA) and iterative-DDA were used for the acquisition. Suspect screening and data evaluation was performed using different spectral libraries before and after aligning the annotated results to a suspect list of chemicals of emerging concern.

This study shows different data processing strategies that can be used for EDA in complex biological samples. Alignment of the results with suspect lists prior the data (MSMS) evaluation can be useful to determine whether some active fractions contain coeluting exogenous and endogenous chemicals. In addition, an alternative data acquisition approach to increase the sensitivity of the chemical analysis is also proposed.

### 3.09.B.T-03 Identification of AhR and ER agonists in eggs of black-tailed gulls in the West Sea Coast of Korea using effect-directed analysis

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In this study, major aryl hydrocarbon receptor (AhR) and estrogen receptor (ER) agonists were identified in eggs of black-tailed gulls collected from Nando Island (n=3) and Gyeongnyeolbi-yeoldo Island (n=2) in the West Sea Coast of Korea, utilizing effect-directed analysis. Results of the bioassay, conducted using H4IIE-*luc* and T47D-*kbluc* cell lines, revealed that AhR-mediated potencies were relatively high in the mid-polar fraction, while ER-mediated potencies showed a significant response in the polar fraction of the egg extracts. Full-scan screening was performed on the mid-polar and polar fractions using GC-QTOFMS and LC-QTOFMS, respectively. A five-step selection process was applied to identify AhR agonist candidates in the mid-polar fraction. In addition, ER agonist candidates in the polar fraction were selected through a six-step criteria application. A total of 25 AhR and 22 ER agonist candidates were identified in the mid-polar and polar fractions, respectively. Among these, three AhR agonists (ethyl benzoate, 3-phenylpropanal, and 1,4-dicyclohexylbenzene) and one ER agonist (hydroxyvalerenic acid) were newly identified. Ethyl benzoate, 3-phenylpropanal, and hydroxyvalerenic acid were natural products, while 1,4-dicyclohexylbenzene was derived from combustion sources. Targeted 27 AhR agonists accounted for approximately 67% of the total AhR-mediated potencies in the mid-polar fractions. Of these, 20-methylcholanthrene (mean=36%), benz[*b*]anthracene (mean=16%), and 10-methylbenz[*a*]pyrene (mean=7.5%) contributed significantly to the AhR-mediated potencies in the mid-polar fractions. Meanwhile, targeted 7 ER agonists contributed 12–95% (mean=53%) of the total ER-mediated potencies in the polar fractions, with estradiol being the major ER agonist (mean=48%), followed by estriol (mean=4.2%) and hydroxyvalerenic acid (mean=0.58%). Further studies are needed to investigate the maternal transfer mechanism according to the partition coefficient of AhR and ER agonists in egg samples. In addition, it is crucial to identify other key factors that determine the maternal transfer characteristics of these substances.

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### 3.09.B.T-04 A Data-Derived Reference Mixture Representative of European Wastewater Treatment Plant Effluents to Complement Mixture Assessment

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Aquatic environments are polluted with a multitude of organic micropollutants, which challenges risk assessment due the complexity and diversity of pollutant mixtures. The recognition that certain source-specific background pollution occurs ubiquitously in the aquatic environment might be one way forward to approach mixture risk assessment. To investigate this hypothesis, we prepared one typical and representative WWTP effluent mixture of organic micropollutants (EWERBmix) comprised of 81 compounds selected according to their high frequency of occurrence and toxic potential. Toxicological relevant effects of this reference mixture were measured in eight organism- and cell-based bioassays and compared with predicted mixture effects, which were calculated based on effect data of single chemicals retrieved from literature or different databases, and via quantitative structure-activity relationships. The results show that the EWERBmix supports the identification of substances which should be considered in future monitoring efforts. It provides measures to estimate wastewater background concentrations in rivers under consideration of respective dilution factors, and to assess the extent of mixture risks to be expected from European WWTP effluents. The EWERBmix presents a reasonable proxy for regulatory authorities to develop and implement assessment approaches and regulatory measures to address mixture risks. The highlighted data gaps should be considered for prioritization of effect testing of most prevalent and relevant individual organic micropollutants of WWTP effluent background pollution. The here provided approach and EWERBmix are available for authorities and scientists for further investigations. The approach presented can furthermore serve as a roadmap guiding the development of archetypic background mixtures for other sources, geographical settings and chemical compounds, e.g. inorganic pollutants. The study is published in *Environment International* (<https://doi.org/10.1016/j.envint.2023.108155>).

### **3.09.B.T-05 Predicting, diagnosing and reducing biodiversity impacts of chemical pollution in surface waters**

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Environmental quality is not sufficiently protected due to a lack of predictive approaches to anticipate exposures to unintended mixtures. Such an approach would fill the gap between prospective, generic per-chemical safety assessments (as under REACH) and retrospective environmental quality assessment (as under the Water Framework Directive). This presentation was triggered by extensive diagnostic investigations in European and Dutch surface water systems, showing that unintended mixtures are a major driver of water quality deterioration. Apparently, the dual approach of prospective and retrospective approaches currently in place is insufficient to reach the aspired 'toxic-free' environment goal. Further consideration showed region-specific prospective approaches that consider regional unintended mixtures exposures and risk to be lacking. Hence, based on observed exposures and impacts of mixtures in contemporary surface waters, this presentation employs a 'model train'-approach, with step 1 considering land use, step 2 emissions of chemicals, step 3 regionalized predicted environmental concentrations and step 4 integrated mixture risks. The results appear useful in two major ways. First, prospectively, current and future land uses can be evaluated regarding potential harm to water quality, resulting in opportunities for pro-active, preventive management. Second, the land-use-related chemical footprint of a study area can be 'traced back' to dominant sources. The dual utility offers an important extra tool to protect and restore water quality. The presentation will show how the model train is built, and how it yields spatio-temporally explicit exposure and mixture risk patterns for chemicals of emerging concern (CECs).

### **3.09.P Complex Mixtures of Chemicals in the Environment and the Human and Eco-Exposome – Next Generation Monitoring (NGM), Toxicity Driver and Source Tracking to Meet Regulatory Needs**

#### **3.09.P-We162 Ad-hoc assessment of non-target screening data for regulatory water monitoring of the future**

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The Green Deal's Zero Pollution Ambition aims for a non-toxic environment and, together with the new Chemicals Strategy for Sustainability, will ensure a sustainable chemicals market that protects human health and the environment from hazardous substances. However, regulatory monitoring programmes in support of environmental, emissions and chemicals legislation rely on conventional target analyses that cover only a fraction of the marketed chemicals. To achieve the goals of the European Green Deal, regulatory agencies need to implement a more comprehensive assessment of chemical mixtures in the environment. Non-Target Screening (NTS) using high-resolution mass spectrometry (HRMS) is increasingly being used for regulatory monitoring of organic pollutants as it facilitates the systematic characterisation of chemical mixtures and identification of previously unknown compounds. At the German federal level, national and state laboratories collaborate via a

database called NTS-Portal (developed by the Federal Institute of Hydrology (BfG)), bringing together HRMS data from different laboratories. The NTS Portal currently contains data from several federal states, the daily water measurement station of the BfG and suspended particulate matter samples of the German Environmental Specimen Bank (2005-2021). The data is structured in two layers, one for the discovery and analysis of unknown HRMS features and one for securely annotated features using a reference library to bridge the gap to conventional target analysis (Database Assisted Screening). An upcoming challenge is the regulatory assessment of HRMS datasets as guidance for the use of semi-quantitative data is missing. Furthermore, there is no central database that provides information on all marketed chemicals including their regulatory status, physicochemical (PC) properties and hazard data. As a regulatory agency, UBA has access to the hazard endpoints provided in the registration dossiers and to emission data of relevant industries. We aim to integrate these data in the NTS Portal, and provide prioritisation and assessment options as well as statistical approaches to identify relevant sources. We have identified 26,470 chemicals listed in the EU chemicals legislation and will enrich the data with PC properties and hazard data from a UBA database and other sources. Furthermore, we provide prioritisation options and give examples for the assessment of typical and atypical chemicals and mixtures.

### **3.09.P-We163 Advancing Environmental Monitoring: Promising Insights into Non-Target and Suspect Screening**

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Non-target analysis (NTA), a qualitative analysis method, aims to identify both known and unknown substances by comparing molecular and fragment masses with internal and external databases. Suspect screening allows to search for substances of interest in an environmental sample. Monitoring projects using NTA and suspect screening methods are presented to give an overview of promising applications.

- NTA to assess the potential impact of the pandemic measures by analysing organic substances before and during the first Austrian Covid19 lockdown in 2020 in wastewater: A total of 440 different substances were detected, with differences found before and during the lockdown, especially in pharmaceuticals and drugs and their metabolites.
- NTA as a tool to investigate the elimination of organic substances in wastewater treatment plants (WWTP): The use of NTA to analyse influent and effluent samples from WWTP demonstrated that ~ 1/3 of organic substances were eliminated. However, ~1/3 of the substances remained, highlighting the need for further treatment improvements. Approximately 47% of pharmaceuticals and their metabolites were still detected in the effluent.
- NTA to perform chemical source tracking of industrial wastewater: Examining modern approaches to address the challenge of complex industrial wastewater discharge.
- Advancements of NTA and suspect screening methods for better identification of emerging contaminants: Internal identification databases are extended continuously for identification (high confidence level) of emerging contaminants, for example the antioxidants para-substituted phenylenediamines (PPDs), namely IPPD, 6PPD, 7PPD, 77PD, 8PPD, and their highly fish toxic quinones.
- Usage of NTA identified substances as dataset for identifying possible river-basin specific pollutants in surface waters: NTA identified substances in industrial wastewater are used as basic data to establish a prioritized scheme for river basin specific pollutants.

Future developments in these modern monitoring techniques include securing financial support for future and retrospective analysis, establishment of in-house digital monitoring, trend recognition, development of an early warning system, focusing on volatile substances as well as on polar substances and supporting risk management strategies and regulatory measures to better protect the environment by evidence-based information.

### **3.09.P-We164 Harmonized LC-HRMS Non-Target Screening in the International Regulatory Framework of the River Rhine Catchment Area**

*Pavel Ondruch, International Commission for the Protection of the Rhine (ICPR), Germany*

The Convention on the Protection of the Rhine is a framework for collaboration among the governments of Germany, France, Luxembourg, the Netherlands, Switzerland, and the European Community to protect the Rhine ecosystem. This legal foundation of the International Commission for the Protection of the Rhine (ICPR), outlines measures to ensure sustainable development, water quality preservation, flood prevention, and ecosystem restoration. The collaborative efforts have led to significant improvements of water quality. Nevertheless, the large number of mostly low concentrated emerging pollutants (EPs) from point and diffuse sources remains challenging. This challenge is regularly addressed, amongst others by the EU Water Framework Directive (WFD) and the Extended Rhine Convention signed in 1999. Especially the program "Rhine 2040" focuses on addressing global environmental challenges, particularly concerning climate change's impact on water management and quality. It aims to ensure sustainable and climate-resilient management of the Rhine River. Additionally, the program emphasizes continued monitoring, collaborative projects. In this framework, the Rhine Project NTS (2021-2024) initiated with the aim to assure NTS data comparability allowing faster information exchange across environmental authorities about increasing number of EPs. In this project a centralized system for fast processing, evaluation and storage of NTS data was developed and applied in daily routine of international monitoring stations of the River Rhine.

The system, called “the NTS Tool”, provides:

- a) Centralized processing with shared databases allowing for more comprehensive screening of EPs (currently >1450)
- b) Near-realtime aggregation of data over the river’s course supporting the classification and identification of sources of Eps
- c) Assistance in the identification of unknowns by supporting knowledge exchange

This NTS Tool will contribute to the fulfilment of the objectives declared in the “Rhine 2040” programme such as the MICROMIN system aiming the reduction of the influx of micropollutants from municipal wastewater systems, industry, and agriculture by at least 30% compared to the 2016-2018 period across the catchment. In the NTS Follow up Project (2024-2029), the NTS Tool will be adapted for early warning of known and unknown substances and enhance the water supply protection. The NTS Tool will help to achieve goals specified in mentioned programs, agreements and the WFD.

### **3.09.P-We165 Improving the monitoring of water bodies through the use of LC-HRMS from the perspective of state environmental monitoring**

*Klaus Furtmann and Susanne Brueggen, LANUV NRW*

The main task of the North Rhine-Westphalia Agency for Nature, Environment and Consumer Protection (LANUV NRW) within the framework of statutory water monitoring are, the monitoring of wastewater, groundwater and surface waters in accordance with various regulations (Water Directive, Wastewater Ordinance, ...) by (target-) analysing up to 600 individual substances at about 3.000 monitoring sites. This target-based monitoring currently requires the application and maintenance of several different methods, each with complex quantification and quality assurance.

As this traditional approach does not provide any information on other potentially relevant substances beyond the specified list of substances, suspect and non-target analyses based on LC-HRMS have been added to the portfolio since 2014.

In future, LC-HRMS will be used for quantitative (targets) and qualitative analyses. In addition, a digital sample archive for retrospective analyses is created for each measuring point.

The change from conventional target analysis with many individual methods that are complex to maintain to the use of a single LC-HRMS measurement, which is subsequently evaluated in different ways (target, suspected target, non-target), holds considerable potential for making water monitoring more effective. However, there are still a few hurdles to overcome before the system can be completely changed:

- Unfortunately, the current version of the Water Framework Directive does not describe how to handle qualitative data.
- The new technology generates very large amounts of data that need to be processed and archived properly.
- The use of the techniques by several users, e.g. along watercourses, requires careful methodological coordination to ensure that comparable data is generated.
- The number of analysis results per sample will increase significantly. New evaluation concepts must be developed that allow the environmental administration to handle the results correctly and implement them in measures.

This concept could change water monitoring and assessment and make it much more efficient without losing information. There is a chance to measure less but learn more about the water bodies.

### **3.09.P-We166 High-Resolution Mass Spectrometry and Nontarget Screening combined to High-Throughput Toxicity Assessments to Address Community Concerns Related to Organic Contaminants in Surface Water**

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Decades of industrial activity led to the designation of the lower 10 miles of the Willamette River as a Superfund Site by the US EPA in 2000. Surface water monitoring and remediation efforts at this site have traditionally focused on legacy pollutants. Community organizations have expressed concerns about overlooked and emerging contaminants in this site. We employed a community engaged approach and selected six locations in the Willamette River for monthly surface water sampling over a 6-month period. Locations above and below the designated Superfund Site were also sampled. We utilized ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry for water analysis. Samples were analyzed using both reverse phase and hydrophilic interaction chromatography to cover a broad range of organic contaminants.

Chemical data was processed using suspect and nontarget screening methods, allowing for the detection of compounds not routinely monitored. Multivariate statistical approaches were used to describe trends and prioritize chemical features for further identification and testing. Organic chemical profiles significantly differ among sites, with Site 6 being the most divergent among them. These differences in the chemical profile may be due to the proximity of Site 6 to the confluence with the Columbia River and a wastewater treatment plant. Clustering analysis revealed that collection month was more relevant than location in describing the differences between samples. The overlap among the annotated compounds each month was lower than hypothesized. Using our *in-house* library and the NORMAN suspect list exchange we annotated 1,025 organic compounds, including pesticides, pharmaceuticals, natural toxins, and food additives. We utilized the high-throughput embryonic zebrafish platform to assess the bioactivity and potential toxicity of collected water samples. Using the Hazard

Comparison Dashboard, we assessed toxicity previously reported for the annotated compounds. The combination of suspect and nontarget chemical analysis with high throughput *in vivo* and *in silico* toxicity testing is a novel approach for the prioritization of water contaminants and further assessment and monitoring. Our results can help inform community groups and regulatory agencies about the hazards associated with emerging water contaminants and the potential risks of exposure from surface waters in the Portland Harbor and other Superfund Sites.

### **3.09.P-We167 Non-Target Screening of Surface Water Samples to Identify Exposome-Related Pollutants: A Case Study from Luxembourg**

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Non-target screening of surface water samples collected over an extended period enables the identification of temporal patterns in exposome-related pollutants. This approach, combined with geographical data on pollution sources, chemical classification, and flow paths, provides valuable insights into the origins and potential risks associated with tentatively identified chemical compounds. In this study, 271 surface water samples from Luxembourg were analysed using high resolution mass spectrometry, complementing routine target monitoring efforts in 2019-2022. Data analysis was performed using the open-source R-package 'patRoom' with a customized non-target workflow. Various scoring terms and identification levels were used to prioritize tentative identifications based on factors such as spectral similarity. Additional database information (*PubChemLite*) and classification software (*classyFire*) were utilized to assess the overall threat posed by the tentatively identified chemicals and prioritize them for future confirmation through targeted methods. The study tentatively identified 378 compounds associated with the exposome, including benzenoids or organoheterocyclic compounds (11 *classyFire* superclasses, 50 sub-classes). The analysis of these compounds revealed temporal variations, with a notable increase in numbers of agrochemicals in May to July. The prevalence of pharmaceuticals such as tramadol in surface waters was also observed. Furthermore, the study investigated potential sources of pollutants by considering common uses and geographical information. The commercial uses of the identified chemicals were largely known (almost 100%), indicating potential contributions from sources like the metallurgic industry and household products. The findings of this study complement existing knowledge on the pollution status of surface water in Luxembourg by tentatively identifying chemicals of concern for potential future inclusion in targeted monitoring methods following additional confirmation and quantification efforts.

### **3.09.P-We168 Exploring the environmental risk of pesticides and transformation products in agricultural area using extensive LC-HRMS screening**

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The intensive use of pesticides and the formation of active transformation products (TPs) are of increasing concern as they are extensively detected in agricultural areas. A comprehensive screening approach is needed to profile pesticides and TPs in watershed near agricultural fields. In this study, an extensive screening method using LC-HRMS was developed not only for 328 targets but also for suspects and unexpected substances. To enrich samples, the solid-phase extraction method using a multi-layer cartridge was utilized. High-frequency composite sampling was performed to explore time-dependent contaminant profiles during 12 stormwater and 7 dry events. Consequently, 21 pesticides and 2 TPs were recognized as major pollutants in the study field. The priority pesticides included bromobutide with the highest mean concentration of 1,800 ng/L, followed by bentazone (910 ng/L), and imidacloprid (560 ng/L). Gaussian curve fitting demonstrated a significant relationship between the pesticide application date and the concentrations of the major pesticides (slope: 0.8365,  $R^2$ : 0.8634). The occurrence trend of pesticides during stormwater event was distinguishable from their TPs, resulting in the identification of clothianidin as TP of thiamethoxam. Additionally, suspect and non-target screening revealed 8 pesticides and 49 TPs. Several TPs (e.g., metolachlor-ESA, thiacloprid-amide, 2-amino-4,6-dimethoxypyrimidine) exhibited more frequent and intense detections compared to their parent compounds. Following European Directive 2013/39/EC criteria (100 ng/L), typically, pesticides showed higher concentrations at stormwater events (51.8%) than dry season (33.6%). Among them, thiacloprid and penoxsulam exceeded criteria only during storm events. This result suggests that the occurrence profile of pesticides and TPs in agricultural fields is highly affected by the application period. To assess environmental risk for pesticides and their TPs, toxicity values were retrieved from environmental quality standards, regulatory acceptable concentrations, and ECOTOX experimental data. For 2 pesticides (i.e., clothianidin and chlorantraniliprole), acute toxicity criteria exceedances above 50% were detected. Especially, the high RQ estimate for clothianidin indicates the potential hazard to aquatic organisms in adjacent streams.

### **3.09.P-We169 Identification of source-specific pollutant patterns and possible indicator parameters of particulate matter emissions from rail transport**

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Since 2021, the German Centre for Rail Traffic Research (DZSF) has been operating an environmental monitoring network along railroads in Germany by investigating the source-related pollution via various entry paths into different environmental

compartments. The investigations focus on seepage water, surface water, groundwater and soil. In addition to engine-related and operational emissions, the need for robust information and studies on abrasion-related emissions has also increased due to possible legal regulations. The primary sources are brake abrasion, rail abrasion, and to a lesser extent, wheel and contact wire abrasion. The aim of the current study is to identify abrasion-related pollutant patterns and the associated elementary indicator parameters of rail traffic in different environmental compartments. For this purpose, extensive test bench and field studies on particulate air pollutants and abrasion emissions were carried out and compared with the results of the environmental monitoring network. The particulate matter emissions were collected on quartz fibre filters (PM10) at a brake test bench and at two field locations (open track and marshalling yard). The measurement setup of the field sites consists of four sampling points (cross-sectional profile and luv-lee-concept) with a measurement duration of several months each. The elemental composition is characterized using inductively coupled plasma - mass spectrometry. In addition, soil samples at different depths and at different distances from the track-axis, precipitation-dependent seepage water samples as well as surface and groundwater samples were taken and analysed at the DZSF environmental monitoring sites. All analyses and sampling were carried out using standardized methods. The results show that both, the particulate matter filter samples from the air monitoring and the environmental samples, have a comparable element composition, particularly in the upper soil layers. In addition, a clear zoning of the heavy metal contamination from the center of the track to the peripheral areas can be demonstrated. The combination of test bench and field tests with different dispersion modelling approaches enables a deeper understanding of the dispersion of pollutants along track systems. The investigations form the first step towards an adapted environmental risk assessment of railroad tracks and the identification of possible indicator substances from abrasion processes in rail traffic.

### **3.09.P-We170 Identifying Source-Specific Contaminant Fingerprints in Waste and Surface Water from High Resolution Mass Spectrometry Data**

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The increasing presence of chemicals in our environment poses a significant risk to both humans and the ecosystems in water and soil. The extensive abundance of substances available can lead to complex chemical combinations affecting both people and organisms, potentially causing harm to human health and ecosystems. Therefore, this study aimed to investigate these complex mixtures, establish connections between their chemical compositions and their origins of pollution and pinpoint unique contaminant patterns specific to different sources. Employing a comprehensive approach using statistical analysis and machine learning, the study analyzed the chemical composition of many wastewater samples from different sources to identify source-specific contaminant fingerprints associated with each source, enabling the prediction of where contaminants originate from.

Wastewater samples were collected from eight distinct sources: food industry, healthcare, landfills, laundries, metal industry, snow, stormwater and vehicle industry. These samples were subjected to analysis using LC-HRMS technology and a variety of analytical screening techniques to decode their chemical compositions. Following this, statistical methods were utilized to examine the variations in chemicals among the different sources, identify the compounds most associated with each source and isolate compounds suitable for predictive analysis. Subsequently, machine learning models were developed to classify and predict the source of each sample. The validity of the models was confirmed using the leave-one-out cross-validation method and the performances of the models were assessed based on their accuracy, precision, recall and specificity.

The top-performing model accurately predicted the sources of 88% of the samples. Some samples showed higher accuracy in predicting their sources, like stormwater (100%), metal industry (92%) and food industry (89%), while others like landfills had a lower accuracy at 71%. This suggests that sources with higher prediction accuracy display a more unique chemical pattern specific to their source, making their identification comparatively easier than those with lower prediction accuracy.

These findings show that certain sources release distinct chemical traces into the environment, highlighting the need to identify these patterns for better monitoring. This is critical, especially in detecting harmful compounds affecting human health and ecosystems.

### **3.09.P-We171 What Does Brake Abrasion in Rail Traffic Consist of? – Physical and Chemical Characterization of Different Brake Abrasion Samples From Rail Traffic**

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Non-exhaust emissions become increasingly important in the transport sector, particularly as a result of new drive systems. Although rail transport is generally regarded as an environmentally friendly mode of transport, significant abrasion-related emissions are produced during operation. A major source of these emissions are mechanical brakes. Mechanical brakes include disc brakes (sintered, organic brake pads), which are mainly used in passenger transport and block brakes (cast iron, organic brake blocks (LL-, K-blocks)), which are used in freight transport. Organic brakes show improved noise characteristics compared to cast iron brakes, but the exact composition of the used composite materials is currently unregulated and largely



unknown.

In order to be able to identify brake emissions in environmental samples and assess the potential risk to humans and the environment, information on the morphology and chemical composition of the abrasion particles is necessary. Due to the large number of different brake types and their diverse material composition, little information is currently available on the characteristics of these emissions.

Therefore, in this study we analyzed the morphology and chemical composition of brake abrasions of different brake types. To obtain pure abrasion samples, air pollutant measurements were carried out on a brake test bench using disc brakes and block brakes consisting of cast iron, sintered and organic materials. Selected filter samples were analyzed regarding their elemental composition and morphology, using scanning electron microscopy with energy dispersive x-ray spectroscopy (SEM-EDX). First results show that C, O, Na, Mg, Al, S, Ca, Ti, Mn, Fe and Zn could be identified in different samples from block brakes. The material composition of the abrasion emissions of the different brake blocks differ from each other, whereby Fe, C and O could be detected in all samples. In a next step, further chemical analyses (e.g. pyrolysis gas chromatography/mass spectrometry) are planned to characterize the material composition of abrasion emissions from organic brakes. The data collected will be incorporated into an initial risk assessment, the identification of indicator parameters and contribute to a cross-modal assessment of non-exhaust emissions.

### **3.09.P-We172 Assessment of endocrine disruptive potencies of air and dust samples from different indoor environments by a battery of in vitro bioassays**

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Humans are exposed to complex mixtures of chemicals throughout their lives. Besides food, the indoor environment is probably the main source of exposure. Major part of human population spends most of their life in the indoor environment, which, due to limited ventilation and the presence of significant sources of chemicals, makes it very relevant for human exposure and potential adverse health impacts. Moreover, numerous chemicals detected indoors exhibit endocrine-disrupting properties.

To assess the endocrine disruptive potential of indoor chemical mixtures, a series of in vitro bioassays were conducted on both indoor and outdoor air samples, as well as indoor settled dust samples collected from various indoor environments. Human-based reporter gene assays were employed to evaluate the effects associated with endocrine system disruption, including estrogenicity, anti-androgenicity, and AhR-mediated toxicity. The results revealed multiple endocrine-disrupting potentials in the majority of the samples.

To place these findings in context with chemical analysis, the identified potentials were compared with potencies predicted by the iceberg modeling based on the analytical data. However, due to either insufficient toxicity data for the identified chemicals or incomplete coverage of the effect drivers in the chemical analyses, the explanatory power was relatively low for most of the observed effects.

The gaps in pinpointing indoor pollutants responsible for the evaluated effects, coupled with the widespread presence of endocrine-disrupting potentials within indoor environments, raise concerns about potential health risks for the human population in these settings.

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### **3.09.P-We173 Effects of textile wastewater from wet processing using effect-based methods**

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Only 3 % of the water on earth is freshwater, making it a limited resource. Textile wastewater is one of the main factors for surface water pollution, especially in central textile-producing countries such as India. Contributing factors include India's high population density and increasing industrialisation. Currently, water quality assessment of textile wastewater in India is limited to analysing physico-chemical parameters such as pH, biochemical oxygen demand (BOD) and chemical oxygen demand (COD), leading to an underestimation of the environmental risk. If untreated or insufficiently treated wastewater enters the environment, it can have adverse effects on aquatic organisms such as acute toxicity, neurotoxicity and endocrine activity. Therefore, further test systems are needed to assess the complex mixture of components contained in textile effluents and develop better wastewater treatment technologies. In this context, effect-based methods (EBMs) are useful to detect the mode of action of chemicals. Samples of the raw textile wastewater were obtained from the textile industry MS/Rohini, Erode in Tamil Nadu in India. The samples were divided into two fractions via SPE using HLB cartridges. One hydrophilic and organic fraction and one lipophilic and inorganic fraction. The hydrophilic fraction of wastewater often contains pharmaceuticals,

hormones and detergents whereas the lipophilic phase can contain heavy metals. Cytotoxicity of the raw textile wastewater and fractions was tested on cell-based level using the Neutral Red assay. First results show the highest cytotoxicity for raw wastewater and the lowest cytotoxicity for the hydrophilic and organic fraction. Further, endocrine effects will be investigated in the CALUX<sup>®</sup> Assay as well as dioxin-like effects with the micro-EROD assay. In addition neurotoxic effects will be tested in behavioural assays using *Danio rerio*.

### **3.09.P-We174 Aromatic Amine Fingerprints of Different Human Activities From Indoor Environments – Textiles as Passive Samplers**

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Indoor human activities lead to the generation of many contaminants that could end up in grey water and reach surface waters when they are not efficiently removed by wastewater treatment plants. When reaching the receiving waters, they contribute to observed adverse effects including mutagenicity. This poses problems for the drinking water production as well as the aquatic habitat itself. Aromatic amines (AAs), many of which are suspected/known mutagens, have been shown to play a role in surface water mutagenicity, although their emission sources have not yet been fully investigated. It is known that several human activities such as smoking tobacco, meat frying, and hair dyeing generate AAs in indoor environments. Their transport from indoors to surface waters can be one of the sources with a significant contribution to their presence in water bodies. A pathway of indoor-related AAs to surface waters is through adhering to textiles which act as passive samplers and release to grey water by laundry washing of these textiles.

In this study, we aim to obtain chemical fingerprints of AAs linked with well-known AA related indoor activities. For that purpose, we organized a sampling campaign in smoking pubs (n=3) hair salons (n=3) and restaurant kitchens with high meat throughput (n=3), where we hung different types of fabrics (cotton, wool and polyester) representing typical textile surfaces indoors to understand their affinity to AA adsorption. In order to evaluate background AA contamination, households of non-smokers that did not prepare and consume meat were also included (n=3).

Several challenges complicate the extraction of AAs from different fabrics. Specifically, high matrix effects due to additives used on textiles such as waxes and fats, and strong bonds forming between functional groups of fabrics and amines. Considering these aspects, we developed a total extraction method that resulted in characterizing indoor activity-specific fingerprints of AAs from 24-hour exposed textiles in aforementioned sampling locations. Using a target and suspect screening method for AAs known to be linked with these activities, the results allow us not only to characterize the AAs humans are exposed to from indoor environments but also to understand the extent of indoor environments as a source of AAs in surface waters for better mitigation efforts to reduce surface water mutagenicity.

### **3.09.P-We175 Passive Air Sampling Networks Combined with Multivariate Statistics Reveal Widespread Non-Aroclor Polychlorinated Biphenyl Sources to the Atmosphere**

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Polychlorinated Biphenyls (PCBs) are the archetype of a complex chemical mixtures in the environment. Atmospheric PCBs were initially thought to originate from the volatilization of commercial Aroclor mixtures, but there is growing evidence of non-negligible atmospheric emissions of non-Aroclor, i.e., unintentionally produced, PCBs. However, the abundance of congeners with both Aroclor and non-Aroclor origins proves distinguishing and quantifying Aroclor and non-Aroclor sources to be a difficult task, and thus studies have been limited. In particular, a comprehensive source appointment of atmospheric PCBs has yet to be done in Canada. To overcome these challenges, we obtained the PCB fingerprints from PAS networks installed across the coastal regions of Quebec (QC) and British Columbia (BC), Canada. The measured fingerprints were then investigated with hierarchical cluster analysis (HCA), to identify distinct regional PCB sources with characteristic congeneric patterns of Aroclors and non-Aroclors. Urban areas, such as Vancouver, BC; Montreal, QC; and Quebec City, QC, showed high levels of a wide range of congeners suggesting origins of Aroclor use. This is consistent with the region's high population density and industrial history. Non-Aroclor exclusive congeners, e.g., PCB-11, were also found, suggesting the widespread release of non-Aroclor congeners to the Canadian atmosphere. PCB-47, 51, and 68, known byproducts of the decomposition of the cross-linking agent 2,4-dichlorobenzoyl peroxide (2,4-DCBP) used in silicone rubber production, were also found together in multiple regions across Canada. These congeners were also strongly associated with PCB-7 and 25, suggesting that the two congeners are minor but non-negligible byproducts of 2,4-DCBP decomposition. Positive matrix factorization (PMF) was then employed to quantify non-Aroclor PCBs contributions to the Canadian atmosphere and had estimated that at least 25% may have non-Aroclor origins. Of the known non-Aroclor sources, decomposition of 2,4-DCBP has only been previously observed as a source of PCBs to indoor air or as a point source, whereas evidence here suggests that the use of 2,4-DCBP is an ongoing widespread non-Aroclor source in Canada. It is likely that 2,4-DCBP is still prevalent in the manufacturing of commercial

products not just in North America but also elsewhere in the world, despite alternative non-chlorinated cross-linkers being available, such as bis(4-methylbenzoyl)peroxide.

### **3.09.P-We176 Mapping the Gaseous Outdoor Inhalation Exposome: Archetypes of Spatial Concentration Variability of Organic Trace Contaminants in the Atmosphere**

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Whereas inhalation exposure to organic contaminants has been proven to negatively impact human health, knowledge of spatial patterns of such contaminants remains limited. Extracts from a total of 170 passive air samplers, deployed at 119 unique sites across two Southern Canadian regions between 2019 and 2022, were analyzed for 353 organic trace substances. Hierarchical clustering, applied to deployment-length normalized concentrations in units of pg per day of sampling using R, revealed four common types of spatial concentration variability in the outdoor atmosphere. "Point Source" signatures are characterized by elevated concentration in the vicinity of major release locations. A "Population" signature applies to compounds whose air concentrations are highly correlated with population density and is often seen for compounds that originate from the use of consumer products. The "Water Source" signature applies to substances with elevated levels in the vicinity of water bodies, from which they evaporate. Another group of compounds displays a "Uniform" signature, indicative of a lack of major sources within the study area. These spatial patterns can be applied to support the identification of sources, quantify emissions, examine associations with health effects, support environmental justice investigations and facilitate prioritization of chemical feature during non-target analysis. In particular, "Point Source" spatial patterns can serve in the identification of individual facilities or industrial sectors likely to contribute atmospheric emissions. By assuming that the emission intensity of chemicals with "Population" signatures scales spatially with population density, it is possible to apply inverse modeling approaches to the measured atmospheric concentrations to derive population-normalized emission factors. Knowledge of spatial pattern of toxic organic air pollutants can further support environmental justice investigations or epidemiological studies seeking associations with health effects. Finally, spatial pattern in the peak intensity of chemical features detected in the non-target analysis of samples from a PAS network could serve in the prioritisation of those features that are worthy of closer investigation and structure elucidation.

### **3.09.P-We177 A fast and novel workflow for screening smoke from forest fires affecting food quality by SPME-SH-DART-MS/MS**

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Smokes from bushfires can seriously influence the quality of food and beverages in adjacent regions. E.g., in case of bushfire events near vineyards, potentially generated volatile phenols (VP) are monitored via chromatography-based analyses for quality control. Throughput limitations inherent to chromatography-based approaches can lead to analytical bottlenecks when a large sample number needs to be run in a short time period like during harvest. We report a rapid screening method for routinely monitored VP using solid phase mesh enhanced sorption from headspace to a chromatography-free direct analysis mass spectrometer (SPMESH-DART-MS) in a highly automated mode. This workflow provides enhanced data quality, faster results, and improved cost efficiency compared to traditional chromatographic screening approaches.

The screening workflow includes the VP 4-ethylphenol (1), 4-ethylguaiacol (2), guaiacol (3), 4-methylguaiacol (4), and o-cresol (5). Sample preparation (~1.5 hours) was performed for 24 samples in parallel as described in [1]. Following sample preparation and extraction, the SPME sheet was transferred to the automated positioning stage of an EVOQ DART-TQ+ triple quad mass spectrometer. MS/MS parameters were optimized (collision energies, collision cell pressure, and scan speed). Matrix matched calibration QC were analyzed, using d3-guaiacol as IS for all compounds. Regression curves were analyzed at 6 calibration levels in quadruplicate including matrix blanks. Accuracy was assessed using 2 QCs analyzed in quadruplicate.

The automated DART-MS/MS analysis of 24 samples was performed in just 12 minutes. Data processing was performed using as standard MS quantitation software with linear regressions of  $R^2 \geq 0.99$  and recoveries of 90 – 110% at 5 µg/L and 25 µg/L concentration levels. The total workflow time for 24 samples was < 1.5 h. Full workflow simplicity, sample throughput, and data quality meet or exceed the accepted metrics of conventional approaches.

References: [1] Terry L. Bates, Gavin L. Sacks. Rapid headspace solid-phase microextraction sheets with direct analysis in real time mass spectrometry (SPMESH-DART-MS) of derivatized volatile phenols in grape juices and wines, *Analytica Chimica Acta*, Volume 1275, 2023.

### **3.09.P-We178 Leveraging Machine Learning and Multi-modal Analytical Techniques for Enhanced Source Tracking of Microplastics**

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Regulatory interventions in mitigating microplastic pollution face significant obstacles due to the inherent challenges in accurately identifying sources, due to the variety of microplastics and microplastic sources in the environment. To enhance source tracking capabilities, our study introduces a sophisticated computational fingerprinting workflow. This workflow integrates non-targeted data gathered from two analytical techniques—thermal desorption gas chromatograph–mass spectrometer (TD-GC-MS) and high-performance liquid chromatography with quantitative time-of-flight mass spectrometry (HPLC-qToF-MS)—to discern unique chemical fingerprints based on additive compositions. To interpret this rich dataset, an ensemble of supervised machine learning algorithms was applied, including Random Forest, Gradient Boosting, Support Vector Machine, and Penalized-Multinomial Logistic Regression (P-MLR). These algorithms were employed to classify and identify the sources of both store-bought and environmental microplastics in environmental samples from five major plastic product categories: children's toys, construction materials, food contact materials, cigarette tips, and face masks. Using multiple Wilcoxon tests and p-value correction methods, a set of at least 26 tracer compounds was identified, capable of distinguishing between the selected product categories. Among the tested machine learning methods, Random Forest and P-MLR showed the highest prediction accuracy, especially when trained with the composite dataset from TD-GC-MS and HPLC-qToF-MS. Our results underscore the potential of machine learning algorithms in advancing the science of microplastic source identification. The study further elucidates a robust methodology for classifier and feature selection, offering valuable insights for optimizing source tracking efforts based on additive compositional fingerprints. In summary, this research stands as a pioneering effort that fuses artificial intelligence with advanced analytical chemistry methods to provide an innovative approach for tackling the critical environmental challenge of microplastic pollution.

### **3.09.P-We179 “In Vivo” ECOD Assay: A Proxy To Unveil Biotransformation In Sediment-Dwelling Invertebrates.**

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The purpose of this study was to optimize the "in vivo" ECOD assay (7-ethoxycoumarin-O-dealkylation) for sediment-dwelling organisms to assess their biotransformation abilities when exposed to sediment-associated hydrophobic contaminants. The ECOD assay is a powerful tool used to measure Cytochrome P450 (CYP450) activity in aquatic invertebrates. The "in vivo" assay is considered more accurate than the "in vitro" method for quantifying CYP450 activity and provides a time-dependent quantification using live test species. However, "in vivo" ECOD assay has only been performed on a few aquatic invertebrate species, and to our knowledge, it has not been optimized for sediment-dwelling organisms. The principle of the ECOD assay involves exposing the organisms to the substrate 7-ethoxycoumarin, which is metabolized to metabolites that produces fluorescence under specific conditions. The intensity of fluorescence is correlated with the metabolite concentration, allowing for quantification of CYP450 activity. This assay was optimized for *Capitella teleta*, a sediment-dwelling organism known for efficient biotransformation of persistent organic pollutants. Individual worms were exposed to sediment spiked with PCBs or Sertraline, and fluorescence detection was performed at regular intervals. Statistical analysis showed that time and treatment interactions were significant, indicating that the treatments had a significant impact on the results. The optimized "in vivo" ECOD assay successfully quantified CYP450 activity in sediment-dwelling organisms exposed to hydrophobic compounds. The method proved to be effective in measuring CYP450 activity in deposit-feeding organisms and can be used as a powerful tool for assessing the effects of sediment-associated contaminants. Further investigations with different species and contaminants are needed to evaluate the efficiency of this optimization.

### **3.09.P-We180 "Identification of AhR active compounds in sediments of highly industrialized area: Application of effect-directed analysis combined with full-scan screening"**

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Gamcheon Harbor, the largest port city (Busan) in South Korea is surrounded by various industrial complexes, such as a ship repair pier, container yard, building material yard, aquatic product plant, and Busan international fish market. The concentrations of traditional polycyclic aromatic hydrocarbons (t-PAHs, n=15), emerging PAHs (e-PAHs, n=16), and styrene oligomers (SOs, n=10) in the sediments of Gamcheon Harbor were detected at all sites (n=12, S1–S12). Several t-PAHs, e-PAHs, and SOs are known to be aryl hydrocarbon receptor (AhR) agonists. The concentration ranges of AhR-active t-PAHs, e-PAHs, and SOs in sediments of S1–S12 were 44–790, 72–290, and 12–420 ng g<sup>-1</sup> dm, respectively. These results indicated that AhR-active substances might be introduced from surrounding industrial complexes. Effect-directed analysis (EDA) combined with full-scan screening analysis (FSA) was conducted to identify novel AhR agonists in sediments from Gamcheon Harbor. The AhR-mediated potencies were assessed in organic extracts, silica gel fractions, and RP-HPLC fractions of the sediments using the H4IIE-*luc* bioassay. Relatively high AhR-mediated potencies were observed in F2.7–F2.8 (mid-polar, log K<sub>OW</sub> 6–8) and F3.6–F3.7 (polar, log K<sub>OW</sub> 5–7) of S1 and S4. Target AhR agonists accounted for only a portion of induced AhR-mediated potencies (up to 43%). FSA was performed for the F2.7–F2.8 and F3.6–F3.7 of S4 using GC-QTOFMS and LC-QTOFMS, respectively. Through four-step selection criteria, such as 1) all detected peaks, 2) matched with library 3) matching score ≥70, and 4) presence of aromatic ring, triphenylbenzene in F2.7 and trenbolone, daphnoretin, and isorhamnetin in F3.6 were selected as tentative AhR agonists. Among these, daphnoretin and isorhamnetin showed significant AhR-mediated potency.

The relative potency values of daphnoretin and isorhamnetin compared to benzo[*a*]pyrene were  $0.4 \times 10^{-3}$  and  $6.5 \times 10^{-5}$ , respectively. The results of this study are significant that provide useful information for the selection and management of the priority substances in the coastal environments.

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### **3.09.P-We181 Effect-directed analysis of proteins with high antibacterial activity. A proof-of-concept.**

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The increasing prevalence of antimicrobial-resistant pathogens has become a global public health concern. Therefore, in the search and development of new antimicrobial agents, the oceans become an attractive source. With the aim of identifying new proteins with antimicrobial properties of marine bacteria origin, a new application based on effect-directed analysis (EDA) is proposed in the present work. Based on the conventional EDA scheme, a new methodology readapted for the identification of proteins in exudates of marine bacteria is proposed. The aim of this work has been to develop both the fractionation and the subsequent proteomic analysis for the identification of new antimicrobial agents, replacing the bioassays by evaluation of antimicrobial activity. In previous studies, the well diffusion test was optimised as a method for the detection of antimicrobial activity in marine bacteria. Once the activity had been detected, in order to simplify the exudate's complexity, fractionation was carried out with a high-resolution size exclusion column and the antimicrobial activity was re-evaluated in each fraction. Finally, once the active fraction was identified, the fraction was analysed by ultrahigh-performance liquid chromatography coupled to high-resolution tandem mass spectrometry in order to identify the protein or peptide responsible for the antimicrobial activity. A first study was conducted to confirm that the new antifungal property detected in the exudate of the marine bacterium *Marinomonas mediterranea* originates from the same protein as the antibacterial property. As seen that the developed method is applicable to another type of marine bacterial exudates, its application has been extended to the identification of antimicrobial agents from the Marine Microbial Collection of the UPV/EHU, which have already demonstrated both antibiotic and antifungal activities of clinical interest. This work shows an extended approach to effect-directed analysis focused to the identification of proteins with a high bioactive role such as the antibiotic and antifungal activity. Size exclusion chromatography with rapid antibiograms and LC-HRMS were successfully combined to demonstrate the affordability of this approach.

### **3.09.P-We182 Assessment of sediment-associated marine microalgal toxicants in industrialized area, South Korea: Application of effected-direct analysis using microalgal bioassay with multiple endpoints**

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Microalgal toxicants in marine sediment from industrialized areas in South Korea were identified using effected-directed analysis (EDA). In the first study, EDA was performed to use microalgal bioassay. It was conducted with three marine microalgal (*Dunaliella tertiolecta*, *Isochrysis galbana*, and *Phaeodactylum tricorutum*) and utilized multiple endpoints using flow cytometry. In sub-fractions of S1 sediment raw extracts (RE), the most potent fractions at three species were only F2.6 (non-polar, Log KOW 5–6) based on growth inhibition. However, the cell viability endpoints were affected in more fractions, including F2.6–7 (non-polar, Log KOW 5–7) and F3.5–6 (polar, Log KOW 4–6). Among the cell viability endpoints, esterase activity seemed to be more sensitive, cell membranes and Chl. *a* were similar, and cell size and intracellular complexity indicated more insensitive than growth inhibition. In the second study, we combined full-scan screening analysis (FSA) to identify unknown microalgal toxicants. In Ulsan Bay, the concentration of targeted compounds could not be sufficiently explained by toxic responses, indicating the presence of unknown toxicants. From the FSA, thirty-one compounds were selected as candidates toxicants. Seven commercially available compounds were confirmed for distribution in Ulsan Bay. Among them, 2-Nitrophenol, 3-Nitrophenol, and 4-Nitrophenol were suggested as unmonitored algal toxicants. In the third study, we performed EDA with improved microalgal bioassay and FSA. The result of exposed sediment extracts and silica gel fractions from Gamcheon Harbor showed differences between microalgal species. The *D. tertiolecta* was more sensitive at F2 and *P. tricorutum* is F3. It seems to be different type of cell membrane (*D. tertiolecta*: glycocalyx-like cell membrane, *P. tricorutum*: thick and hard cell wall). Result of FSA, the 8 microalgal toxicants were confirmed. And, the EC50 of newly microalgal toxicants in sediment from Gamcheon Harbor was suggested. Identified unknown microalgal toxicants were performed successfully using EDA. Microalgal bioassay combined cell viability with flow cytometry provided more sensitive and detailed physiological responses, which do not allow traditional endpoints. Unknown microalgal toxicants were successfully identified by use of EDA with FSA. In further study, the distribution, source, fate, and ecotoxicity of newly identified microalgal toxicants need to investigate.

### **3.09.P-We183 Developing an Effect-Directed Analysis Approach for Prioritizing CECs in Urban Groundwater**

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Climate change exerts growing pressure on freshwater resources, particularly in southern Europe, where droughts are projected to be more frequent, intense and long. In the metropolitan area of Barcelona (Spain), the underlying urban groundwater (UGW) may serve as an alternative freshwater resource during these dry periods. However, its use may be hampered by the presence of a wide range of contaminants of emerging concern (CECs) which may pose risks to human health. A proper assessment of the groundwater quality in Barcelona is therefore necessary to ensure the safety of this potential drinking water resource. The primary objective of this work is to identify CECs that can be the main drivers of toxicity in UGW. To do this, an effect-directed analysis (EDA) approach using a zebrafish embryo bioassay will be developed as a strategic prioritization tool to identify CECs with relevant human health effects. In this approach, transcriptional alterations will be used as the basis for evaluating the toxicity of UGW samples and their fractions. Suspect and nontarget screening will be performed for the identification of CECs associated with the bioactive fractions, and when the key toxicants have been identified, target analysis will be conducted to quantify their concentrations in the aquifer. The EDA workflow developed in this project has the potential to be applied in the effect-based monitoring of other aquatic systems, and the results obtained in this study will be useful in contributing to a watchlist of priority substances in groundwater.

### **3.09.P-We184 Effect Assessment Of Urban Waters In Malawi And Identification Of Effect Drivers Using Pull-down Assay Coupled To Non-target Mass Spectrometry Analysis**

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Water contamination with a broad spectrum of micropollutants originating from wastewaters, industry and agriculture is a global environmental issue, however, most research addressing this theme was performed in developed countries. Data on water pollution from developing countries are scarce despite of pressing need to introduce measures to ensure satisfactory water quality in poor regions with quickly growing populations suffering from multiple natural and socio-economic pressures.

Urban river water samples were collected with passive samplers at two streams draining a large high-density urban settlement (Ndirande) in Blantyre, Malawi, to compare chemical and effect profiles to the situation in Europe, and between dry and wet seasons typical for Sub-Saharan Africa. A battery of *in vitro* bioassays targeting endocrine endpoints was used to characterize the overall effects of compounds contained in the highly complex water extracts. We detected several effects i.e. estrogenicity, androgenicity, dioxin-like activity, agonism on thyroid hormone receptor and inhibition of binding to transthyretin protein (TTR). There were only minor differences between locations and seasons and we selected the TTR binding inhibition endpoint for detailed investigation to identify the causative compounds.

To this end, novel pull down assay approach based on highly specific protein-ligand interaction was used for separation of active ligands from the complex environmental extracts. Eluted ligands were subjected to non-target screening using a two-stage workflow. HRMS (High resolution mass spectrometry) full scan in ESI<sup>-</sup> and ESI<sup>+</sup> (electron spray ionization) was acquired. Full scan data were processed by CompoundDiscoverer 3.3 workflow consisting of differential analysis to identify suspect signals for consequent LC – full scan MS1 combined with PRM (parallel reaction monitoring) MS2 acquisition. There were 40 compounds bound selectively to pull down protein at all investigated localities. On the other hand, there were about the same number of compounds specific only for one combination of site and season. Natural and synthetic chemicals were identified among pulldown-selected compounds at various Schymanski levels. Despite different pollution profiles and development levels, some highly active TTR compounds were identical to those in European waste and surface waters. The project has received funding from the Czech Science Foundation (GACR) under grant agreement GX20-04676X.

### **3.09.P-We185 Effect-directed Analysis of Belgian Municipal Wastewater Treatment Plant Effluent Using Cyanobacteria *Microcystis Aeruginosa***

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Municipal wastewater treatment plant (WWTP) effluents are a primary source of organic micropollutants (OMPs) in the aquatic environment. Pesticides, industrial chemicals, human drugs, and antibiotics are examples of OMPs that have low removal efficiency with conventional wastewater treatment technologies. This could lead to a harmful impact on aquatic wildlife. Therefore, identifying the main toxicity drivers is imperative for developing advanced wastewater treatment technology to eliminate such risks. However, this identification process is challenging due to the myriad of OMPs and their transformation products in the environmental samples and the limited analytical capacity to detect trace concentrations of these compounds. Effect-directed analysis (EDA) is a useful approach that involves effect-based monitoring and fractionation to reduce the compound mixture complexity before identifying compounds of environmental relevance. In this study, 50 L of a secondary WWTP effluent from a major city in Flanders, Belgium, was sampled and extracted using large-volume solid phase

extraction with Chromabond HR-X. *Microcystis aeruginosa* was selected to assess toxicity according to OCED201 guidelines for 96 hours of exposure due to its sensitivity to antibiotics and herbicides. This effluent extract revealed a growth inhibition to cyanobacteria with the EC10 equal to a relative enrichment factor (REF) of 6.8. The sample was fractionated with reversed-phase high-performance liquid chromatography (RP-HPLC), collecting twenty-eight fractions at two-minute intervals. Subsequently, the fractions were recombined to assess toxicity recovery after the fractionation procedure. Three fractions exhibited significantly different growth rates than the control group, as determined by Mann Whitney test U test ( $P < 0.01$ ). The toxicity was well recovered after the fractionation, with 87% of toxicity recovery. Mass spectrometry analysis is under investigation to identify potentially toxic compounds in these fractions that pose an effect on the cyanobacteria.

### 3.09.P-We186 Event driven taxonomy (EDT): Deep learning links EDA and NTA

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Ecosystems are often exposed to complex mixtures of chemicals. Identifying causative toxicants is essential to mixture risk assessments but there are data gaps among chemicals and bioactivities. In the big data era, scientific decision based on data mining and machine learning has been proposed as a powerful strategy. The collection retrospective risk-related big data can greatly promote scientific decisions on endpoint selection rather than traditional arbitrary decision-making that merely depends on expert judgment. We proposed an event-driven taxonomy (EDT) concept to integrate adverse outcome pathway-based metadata in risk assessments. Using EDT, molecular initiating events and the substances causing the events were fused in a data matrix, which was named an event driver (ED). Results showed narcosis, estrogen receptor- and aryl hydrogen receptor-mediators were the major EDs in aquatic systems across China. Individual regions had distinct ED fingerprints. Subsequently, an EDT-based artificial intelligence-assisted integrated testing strategy (ITS) was constructed for assessing aquatic risk by integrating high-throughput screening bioassays and chemical predictions. This EDT-based ITS was evaluated using complex sediment mixtures eliciting aryl hydrocarbon receptor activation and oxidative stress response. While mixture prediction using expert knowledge-oriented target analysis only explained  $<10\%$  of observed sediment bioactivity, a big data-driven suspect analysis expanded the fraction explained to  $>80\%$ . Additionally, deep learning models were developed to extract structure fingerprints of bioactive suspect candidates and then convert these fingerprints to HRMS-recognizable fragment ions for non-target analysis. The EDT-based ITS tool provides a promising strategy for mixture risk assessments in the big data era.

### 3.09.P-We187 Analysis of polychlorinated n-alkanes (PCAs) in food from the Swedish market

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Polychlorinated n-alkanes (PCA) are the main constituent of chlorinated paraffin (CP) mixtures. PCAs are persistent, bioaccumulative, and toxic. Thus, the use and production of short chain chlorinated paraffins (SCCPs, i.e. PCAs-C<sub>10-13</sub>) were banned in 2017 at the Stockholm convention. Even so, CPs are still produced in large volumes and used in a wide range of applications. Consequently, humans are exposed to these ubiquitous contaminants, and dietary intake is usually the main exposure route. Recently, concerns have been raised about this complex group of compounds, however, there is still a lack of knowledge on their presence, distribution, and temporal trends of dietary intake in order to assess human risks. The current study aims to fill the knowledge gap on PCA levels in food items. In 2015 Swedish National Food Agency (SLV) conducted a market basket (MB) study, in which representative food samples from the Swedish market were analyzed for PCAs. In this follow-up study, we analyzed the MB collected by SLV during 2022, together with three samples from the previous MB of 2015. The food groups with an average consumption of 0.5 kg per person per year or more were included, covering approximately 90% of the total annual consumption, in kg/person. The MB represents more than 130 food items, which were pooled together for each category. A total of 17 different categories were studied, such as dairy, meat and cereals. Samples were extracted following the same method applied in 2015, with minor changes, and analyzed using liquid and gas chromatography coupled with quadrupole time of flight. The instrumental setups allowed to measure  $\sum$ PCA-C<sub>9-30</sub> with 3-14 chlorine atoms. Preliminary results show different homolog patterns dependent on the food groups. This holds considerable significance as the bioavailability of PCAs hinges on the carbon length and chlorination degree. For instance, the  $\sum$ PCAs-C<sub>18-30</sub> in dairy liquid and solids samples was relatively higher compared to the other food categories. PCAs-C<sub>10-13</sub> were present for most of the samples. Moreover, preliminary findings include an increase of  $\sum$ PCAs-C<sub>10-13</sub> for the sweets sample from 2015. Overall, this new study will provide more information regarding estimated per capita exposure to PCAs, as well as their homolog pattern and possible temporal trends. This information is crucial for assessing the risk associated with PCA intake through food.

### 3.09.P-We188 Assessing the Accuracy of Findings in Oil Pollution Cases Using Likelihood Ratios

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The liability of an oil spill has been established in court using chemical analyses as evidence. These analyses, performed on spill and suspected sources samples, rely on identifying the chemical composition of the samples, called oil fingerprints, and comparing them using statistical approaches. The oil fingerprints are expressed as a set of ratios between abundances of oil-discriminating compounds, named diagnostic ratios (DR). The Nordtest method and EN 15522-2 standard are two methods that have been used to compare DR of oil samples under different DR comparison conditions and using distinct statistical

approaches. The Nordtest method suggests using the *t*-Student test (S-t), assuming the normality of the DR probability distributions, whereas EN 15522-2 requires applying a maximum relative difference of 14%, a single criterion (SC) based on the most expectable dispersion for comparison of all DR of the DR set. Assessing DR agreement using parametric statistics that rely on assumptions or approximations, such as S-t and SC, may increase the probability of inaccurate findings. Thus, to ensure the quality of the identifications, new statistically sound approaches to result interpretation must be developed. Furthermore, it is important to present the chemical analysis's weight in court, which is often provided as likelihood ratio (LR). The LR is interpreted as a conditional probabilities ratio that, in the case of oil pollution, evaluates the compositional equivalence claim between samples in relation to the prosecution's and defense's hypotheses, which claim that the polluter is the defendant and the polluter is someone other than the defendant, respectively. Determining LR can be tough and demands the use of statistical models or databases.

This study was applied to spill and suspected source samples from different oils to present a novel approach that produces statistically sound criteria for the DR comparison. The new approach was developed as a computation tool and is based on DR simulation using the Monte Carlo Method (MCM). Using the DR comparison conditions recommended by Nordtest and EN 15522-2 methods, the tool simulates DR for two samples that is used to assess DR equivalence and estimate the probabilities of true and false compositional equivalence claims for MCM, S-t and SC. These probabilities allow us to calculate LR and assess the accuracy of the findings for each oil spill case investigated.

### **3.09.P-We189 Assessing VOC Performance, Perception and Indoor Air Impacts of Commercial Plug-in Fragrance Diffusers**

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Volatile organic compounds (VOCs) are contained in many materials found in the home, including household and personal care products, paints and adhesives and are released from cooking, plants and combustion. Quantifying the air quality effects of a product is complex; it adds incrementally to a pre-existing background of chemicals and is influenced by physical factors including air exchange rate (AER) and deposition. Emissions from product use depend on formulation and on human behaviour. The potential impacts on indoor air quality from the use of plug-in diffusers have been assessed using GC-MS based measurements from 60 homes using controlled experiments with variable room size and AER; human sensory panels to determine perception and potential patterns of use. This is the first such combination of advanced sampling, broad VOC analysis and quantification, combined with non-biased 'normal' residences and the statistical analysis of all product use in the home; followed by increased device use in both home and controlled conditions. A statistically significant increase in alpha-pinene, a major diffuser ingredient, was only detectable in homes with low ventilation rates. Use of test rooms under controlled AER allowed for incremental product addition and a predictive numerical model of concentrations effects indoors to be tested. The effect of additional device use was evaluated by trained panellists showing that beyond 2 devices little benefit was seen indicating that even in abnormal use, VOC levels are likely to be self-limiting as no sensory benefits arise. This work bridges the understanding of real-home exposure to VOCs of all types, demonstrating the added value of study design to better understand and inform the public on exposure levels

### **3.10 Direct and Indirect Impacts of (Nano- And Micro-)Plastics in Terrestrial Ecosystems: Current Status and Future Trends**

#### **3.10.T-01 Towards an improved fate assessment of microplastics: Inclusion of specific analyses and abiotic degradation in regulatory tests**

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Intentionally added microplastics are now restricted by the European Union. Exempt from this restriction are (i.a.) biodegradable polymers. Most of the available test methods to classify microplastics as biodegradable target mineralization (e.g., CO<sub>2</sub> evolution) as pass criterion. However, it is questionable, whether the overall degradation of a polymer is well described by its mineralization. For example, other processes such as abiotic degradation and the release of fragments and transformation products are not addressed. Nevertheless, these changes can have an impact on assessing the environmental fate and toxicity of microplastics. Therefore, in this study we aimed to combine a test for abiotic degradation (photolysis) with one of the available methods for testing biodegradability. Finally, we included analytical tools to improve our understanding on how microplastics degrade.

In this study capsules used in agricultural applications were investigated as <sup>14</sup>C-labelled product. An aqueous suspension was exposed to simulated sunlight. Thereafter, a sequential filtration was performed to identify major alterations in size. Further, the aim was to identify dissolved and more bioavailable molecules released from the capsules (<0.2 μm). Therefore, Liquid Chromatography - High Resolution Mass Spectrometry coupled with radio detection (radio-LC-HRMS) was employed. Lastly,



the biodegradability of the irradiated and non-irradiated suspension was tested by performing an OECD 301B – CO<sub>2</sub>-evolution test in an aqueous medium.

Our findings demonstrate that the size distribution of the suspension was highly effected by the light exposure. The polyurea capsules were “degraded” from approx. 15 µm down to nanoscale. Radio-LC-HRMS analysis enabled to identify the release of one major polymer component (aminocaproic acid). Most likely the release of this molecule was the key driver that lead to a biodegradation test result of 28 % in 28 days.

In summary, when released to the environment, MP are prone to biotic and abiotic degradation. Therefore, it is reasonable to additionally include abiotic degradation as part of regulatory tests, resulting in a more realistic and environmentally relevant assessment. Further, study results revealed that light exposure can largely affect polyurea microcapsules degradation - transformation products and fragments of different polymer sizes were shown to be released whose fate in the environment are still unknown.

### **3.10.T-02 Photolysis on soil surface makes persistent microplastic biodegradable**

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Increasing human population and climate change lead to an urgent need for a more efficient agricultural production to guarantee food security. For instance, advanced plant protection products have been realised by the encapsulation of the active ingredient in a polymeric shell, which can reduce pesticide application loads. Nevertheless, the release of microplastic (MP) into the environment could as well have negative impacts. Therefore, intentionally added MP to products are now restricted, with exemptions for (i.a.) biodegradable polymers. The degradability of MP needs to be tested, however, abiotic degradation is not included in regulatory assessment. Thus, the aim of this study was to combine a phototransformation test on soil surfaces with a biodegradation test in soil. Furthermore, the implementation of a new extraction method from soil is scheduled to get insights on capsule alterations regarding the size in a forthcoming analysis.

Polyurea MP capsules, applied in agriculture, were investigated as <sup>14</sup>C-radiolabeled product. In a first step, the capsules were applied to dry soil and exposed to simulated sunlight. Further, a dark control was prepared at 80°C. This approach aimed to reliably distinguish between degradation induced by thermal effects from photolytic degradation. In a second step, soil aliquots of the first test were mixed into pre-incubated soil for the downstream biodegradation test (OECD TG 307). In both, the light and the biotically induced degradation test, mineralization (<sup>14</sup>CO<sub>2</sub>-evolution) was tracked. The remnants of the polyurea capsules are intended to be extracted from the soil with a newly established oil extraction method using octanol to analyse possible MP fragmentation or the release of transformation products.

During the exposure to simulated sunlight 11 ± 5 % of the total applied radioactivity (AR) was found to be mineralized. The downstream tested biotically induced mineralization reached 11 ± 4 % AR during the first 11 days (to date). Contrarily, a negligible amount of non-irradiated polyurea capsules were mineralized.

The results of this study demonstrate that the (bio)degradability of polyurea capsules is highly effected by photodegradation. To achieve a more realistic fate assessment, abiotic effects such as the photolysis tested here need to be considered to get a more accurate picture of possible pathways that MP can undergo.

### **3.10.T-03 A Two-Year Incubation Study: Investigating the Vertical Transport of Microplastics in Soil and its Impact on Soil Pore Development**

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With escalating concerns regarding sustainable resource management, the influence of microplastics (MPs) on soil ecosystems has become a subject of growing interest. The unintentional introduction of MPs into agroecosystems through various agricultural practices has sparked concerns regarding their long-term implications for soil structure and fertility. This study presents a comprehensive exploration of the influence of MPs on soil pore structure and MPs transport dynamics in agricultural environments. Leveraging the results of a two-year investigation, we assessed how MP morphology (fragments, fibers) plays a role on these aspects in an agricultural field. We installed packed soil columns (9 cm height) with two variations: one without the addition of MPs (control) and another spiked with polyethylene terephthalate MPs (1% w/w Indium doped for tracking) in the top 2 cm. These columns were exposed to natural weather conditions and underwent crop cultivation cycles. Each treatment and timepoint had a minimum of 5 replicates. We monitored the evolving soil pore structure using X-ray computed tomography at the beginning of the experiment as well as at the 1- and 2-year timepoints. MPs transport vertically

through the column was monitored at the same time intervals, where columns were sacrificed at the specified time point, segmented into four, 2 cm sections. MPs were analyzed after homogenization and subsampling of the soil layers and subsequently quantified by Inductively Coupled Plasma Mass Spectrometry, taking advantage of the Indium doping as a proxy for the plastics. The total recovery of the MPs was  $94.7\% \pm 2.5\%$ , suggesting both minimal MPs migration out of the columns and an appropriate sampling strategy. Initial results for the 1-year timepoint suggest that MPs are transported through depth, with 24.8%, 0.9%, 0.5% at 2-4 cm, 4-6 cm, and 6-9 cm depths, respectively. This transport is likely a combination of advective particle transport and bioturbation, both from soil dwelling organisms and plants. Our research delves into the intricate impacts of MPs on soil pore structure, aiding informed decisions for sustainable agriculture and environmental stewardship. By employing a realistic experimental design mirroring natural soil networks and incorporating soil biota, our study sheds light on the enduring consequences of MPs contamination, providing practical insights for sustainable resource management in agricultural ecosystems.

### **3.10.T-04 Microbial activity in field-plot experiment samples (Spain, Germany, and Finland) contaminated by conventional and biodegradable microplastics**

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A significant amount of agricultural plastics introduced into the European market each year ultimately finds its way into the soil, degrading into microplastics (MPs). Besides single species and microcosm tests, field plot experiment studies give us more environmentally relevant results covering different biogeographic regions and climatic zones.

In the field plot experiment study, which ran as a part of the Papillon project (Horizon 2020), the soil was contaminated by two agricultural microplastics: (i) conventional LLDPE (M-rPE-black-A0, P3) and (ii) biodegradable starch-PBAT blend (M-rBIO-black-A0, P4) at environmentally relevant concentrations (0.005 and 0.05% weight of MPs / weight of soil). Five replicates of each concentration and agricultural plastic plus controls (no added contamination) gave 25 field plots, which were settled in spring 2022. Three different biogeographic regions of Europe (Spain, Germany, and Finland) were included to elucidate behavior in soil. The soil samples were taken in two consecutive seasons (autumn 2022 and autumn 2023).

Microorganisms form basal functions in the soil and are crucial for critical processes in the soil ecosystems. Four methods describing N- and C- mineralization were measured for each treatment of the field plot experiment study and in both seasons: basal respiration, substrate-induced respiration, potential ammonification, and potential ammonium oxidation. All methods were measured in a sufficient number of subsamples, and some subsamples were remeasured repeatedly in time to get a time-dependent response. This approach minimized the variability of the measurement.

In Spain, microbial activity was unaffected by the treatment in 2022, but changes were observed in 2023. For all methods measured, significant decreases ( $p < 0.05$ ) of microbial activity were shown for the high concentrations of both conventional and biodegradable microplastics. In other regions, the soil behaved differently. In Germany, the trend was visible only for basal respiration in 2023, where all treatments showed significantly lower values of microbial activity than control ( $p < 0.05$ ). In Finland, significant differences ( $p < 0.05$ ) were found between the substrate-induced respiration of control soil samples and samples contaminated with high concentration of P4, again only in 2023. Different behaviors in different regions strengthen the climate regime's importance and should be considered in agriculture plastic management.

### **3.10.T-05 Transfer of nanoplastics into subsequent plant generations**

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Microplastics including nanoplastics, small piece of plastics less than 1 mm, threaten to soil ecosystems. Plant are producers and food sources, and they can transport microplastics into predators and humans through food chain. Though numerous studies for the effects of microplastics on plants have conducted, the transfer of microplastics into subsequent plant generation are not explored yet. Thus, this study aimed to investigate the parental transfer of microplastics in plants. We exposed 200-nm nanoplastics to pea *Pisum sativum* for about 60 d. The pea fruits (F1) were harvested and were replanted in clean soil for 14 d. After incubation, the collected pea fruits and pea plants were observed by confocal laser scanning microscope. The significant nanoplastic fluorescence was distributed in pea fruits and plant, both. Within F1 pea fruits, fluorescence was located a circular pattern in the embryo area, in contrast, it exhibited scattered in the cotyledon area. Frequency of nanoplastic fluorescence in F1 plants were similarly identified in roots and stems. Nanoplastics were distributed in vascular bundles rather than epidermis, indicating that they were transported from parent plant through circulation, not through external exposure. Our findings presented evidence of parental transfer of nanoplastics absorbed by plants from soil. They provide the possibility for the transport and cycling of nanoplastics within soil ecosystem.

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### 3.10.P Direct and Indirect Impacts of (Nano- And Micro-)Plastics in Terrestrial Ecosystems: Current Status and Future Trends

#### 3.10.P-Tu204 Lateral Microplastic Transport Following Heavy Rainfall Events

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Microplastics (MPs) can accumulate in (agricultural) soils. These soils can either act as a sink or a secondary source to other parts of the environment. Lateral water-driven transport and soil erosion are potentially important processes for MP to enter aquatic ecosystems. However, these transport processes from (agricultural) topsoils are not well understood. To address this knowledge gap, we investigate the transport of MP during heavy rainfall events as a function of slope (1°, 3°, and 9°) and tillage, using eight independent soil-filled containers (80 x 80 x 20 cm). The soil originates from the Riparian Stream Mesocosm facility of the RPTU Kaiserslautern-Landau and is incubated for about eight weeks prior to each experiment and spiked with field-relevant contents (10 g/m<sup>2</sup>, about 70 mg/kg dw) of polystyrene (PS), polyvinyl terephthalate (PET) and tire wear (TW). The studies are based on a rainfall simulation (about 60 mm/h) with spatial homogeneous rainfall coverage of the area (i.e., 80 x 80 cm) using a small-scale irrigation plant. The runoff is collected from each container to assess the transport of each MP. The MPs are either spread onto the soil to simulate worst-case conditions (Runoff Experiment 1) or incorporated into the soil to increase field relevance (Runoff Experiment 2). Our dataset is expected to give valuable insights on the fate of MPs and their transport from soils to aquatic ecosystems, thus indicating exposure risk for different organism groups.

#### 3.10.P-Tu205 MICROPLASTICS DISTRIBUTION AND CHARACTERIZATION IN SOIL, WATER, SEDIMENTS AND PLANTS IN DIFFERENT URBAN AREAS OF SAUDI ARABIA

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More and more researchers are directing their attention to the pollution of water and sediment by microplastics (MPs). However, there is limited understanding of how these MPs are distributed in soil and plants. Hence, the objective of this study is to examine the distribution, morphological features, and physicochemical indices of MPs in different environments within Riyadh, Al-Jubail, and Dammam cities in Saudi Arabia. Riyadh is the capital and largest city of Saudi Arabia whereas Al-Jubail and Dammam are major industrial centres and homes of the largest petrochemical complexes in the world. The MPs were identified by both visual inspection and microFTIR to characterize them chemically. The results exhibited that, water and sediment, soil and plants growing on it have been polluted by MPs. They were present in the form of pellets, fragments, film, fibers, and foam. MPs with 0.05 to 0.5 mm possessed the largest proportion in the water, followed by MPs with < 0.05 mm. However, soils and sediments mainly contained larger-sized MPs, including a high proportion of those ranging between 2 and 5 mm even through the fraction of MPs between 0.05 and 0.5 mm is also very relevant. In water, sediment and soil polyethylene and polypropylene were the most important plastics. The MPs in plants were all small, <0.05 mm, and were mainly composed of viscose fibers and polyethylene. Several endogenous compounds such as cuticle proteins, cellulose, and other plant-characteristic proteins were found in the plants. The MPs found in various compartments highlight that irrigation water can be a source of MPs, but clearly not the only one. Other sources, such as the degradation of plastics and MPs in soils, the application of substances to prevent compaction, or wind and airborne deposition, are responsible for the presence of larger-sized MPs in soils and sediments. Finally, it is evident that plants can absorb MPs. However, in this case, the MPs are always of smaller size (which is logical since they have to traverse several biological barriers). This is coherent with the literature. This study sheds light on the distribution of MPs in the environment, including cities with different characteristics under a desert climate and affected by climate change.

#### 3.10.P-Tu206 Assessing the presence of plastics within the European hedgehogs diet - supplementary food and wild prey

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To understand the movement of plastics through a terrestrial food web, both the European hedgehog's supplementary feed and wild prey species were analysed for potential microplastics. This was achieved by carrying out a survey of 30 rescue centres and testing the top 8 brands of dog, cat and hedgehog foods, which resulted in 48 samples being tested and analysed using micro FTIR. For the wild prey species > 600 samples of 6 different insect groups were collected from 5 different habitat types across Sussex, UK. The samples were analysed for microplastics using micro FTIR. This allowed for an understanding of the plastic content in both types of food and their potential impacts on the European hedgehog.

#### 3.10.P-Tu207 The Combined Effect of Microplastic Exposure (PBAT or PET) and Seawater Inundation on the Coastal Plant *Plantago coronopus*

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Coastal ecosystems face a multitude of pressures including plastic pollution and climate change-driven sea level rise which are predicted to intensify over the coming decades. As such, it is necessary to assess how coastal species may fare under these

changing environments, and how stressors may interact. The effect from exposure to polyethylene terephthalate (PET): a conventional non-biodegradable plastic, and the biodegradable polymer polybutylene adipate terephthalate (PBAT), in combination with seawater inundation on endpoints associated with growth and stress was examined in the coastal dune species *Plantago coronopus*. *P. coronopus* were grown for three-months from seeds (15°C; 18:6h photoperiod, watered 3 times per week), before being transplanted to compost containing PBAT ( $n = 20$ ) or PET ( $n = 20$ ) at 0.02 g/kg (<300µm diameter) and a no plastic control group ( $n = 20$ ). After 35 days, replicates ( $n = 10$ ) from each plastic group were flooded to pot height with artificial seawater for 72 hours and grown for a further 29 days. Throughout the experiment plant mortality, necrosis and photosynthetic efficiency (Fv/Fm) were recorded. Surviving plants were harvested and roots and shoots were separated and dried for biomass determination. There were clear synergistic effects of plastic and seawater on the photosynthetic efficiency of plants. Plants exposed to biodegradable PBAT + seawater, and conventional PET + seawater, had significantly reduced Fv/Fm values compared to all other treatments, with effects most potent in the PBAT + seawater treatment. Plants were able to mediate adverse effects, with no differences in photosynthetic efficiency detected 18 days post-inundation. Seawater inundation was the main stressor explaining the quantity of necrotic tissue, however interactions between microplastics and seawater influenced resource allocation (root:shoot ratio) of plants. Coastal plants have vital roles in preventing erosion and providing food and habitat for a diversity of species. This study demonstrates the complex interactions of exposure to biodegradable and conventional microplastics in combination with seawater flooding events on selected plant endpoints, with potential ramifications on coastal ecosystems.

### 3.10.P-Tu208 Characterization and spatial distribution of mesoplastic particles in an arable soil

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Extraction of plastic particles from soil is challenging and time-consuming and this is one reason why currently exceptionally little spatial information on plastic distribution at the field scale has been gathered. For risk assessment, adequate sampling should complement coherent morphological, physical, and chemical plastic profiling. In this study, we investigated the spatial distribution of mesoplastic (MeP; size > 5 mm) particles in an arable soil (Haplic Cambisol) managed intensively by 12-years of compost application. Geo-referenced samples ( $n = 128$ ) were collected at a three hectare study site in Northern Germany. In addition to the detailed characterization of MeP, the dataset was complemented by the simultaneous recording of soil properties in order to compare dispersion measures of MeP with non-technogenic soil properties such as pH, soil organic matter, cation exchange capacity, and bulk density. In total, we found 259 MeP particles with a predominance of transparent packaging foils and colored fibers, likely due to insufficient separation during recycling of biowaste. Caution is advised when measuring the particle mass. As an example, fibre bundles harbored the largest polymer-associated soil (PAS) mass with, on average, 0.544 mg soil per  $\text{mg}^{-1}$  particle. We recommend using a 0.1 mol L<sup>-1</sup> tetrasodium pyrophosphate (TSPP) solution to purify MeP particles by removing attached soil before weighting and using these mass data for risk assessments. Otherwise, we found a radical overestimation of particle weights retrieved without the cleaning procedure between 160% (films) up to 296% (fragments), and this will bias the results. The MeP count and mass featured the highest coefficient of variation in comparison to soil properties, e.g., pH or bulk density. After removal of the top three extreme values, a pure nugget variogram indicated that no spatial dependency for particle counts existed. In this case, simple random sampling approaches are applicable. Bootstrapping revealed that eight samples are sufficient to estimate mean soil pH with an average error of  $\pm 10\%$  and 95% confidence level, but over five times larger number of replicate samples are expected to determine the MeP polymer mass.

### 3.10.P-Tu210 Microplastic Omnipresence: Automated Detection of Atmospheric Microplastic Deposition in Alpine Environments using $\mu$ FTIR Mapping and Open Specy

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While the presence of microplastics (MPs) in the atmosphere is known, there are few studies that are focused on MP air transport patterns, origins, and deposition rates into terrestrial environments. This study aims to calculate the flux of MP atmospheric particles into terrestrial environments with a novel automated  $\mu$ -Fourier Transform Spectroscopy ( $\mu$ FTIR) technique. This method will transform MP detection workflow by shifting from picking individual MPs to an automated detection of MPs, which can become a standardized method. Additionally, it will allow detection of MPs down to 50 µm and high throughput analysis of spectra. To demonstrate the effectiveness of this method, environmental samples from 15 alpine sites were collected using hydrology snow density measurement approaches. Once the samples were collected, the meltwater was filtered onto gold-sputtered polycarbonate filters. Filters were mapped in reflectance mode using a Nicolet iN10 ( $\mu$ FTIR) with a two-dimensional focal plane array. Once spectra were collected, up to 300,000 spectra were analyzed using the open-source program Open Specy. A novel automated MP identification program and database was developed to identify the shape, polymer type, and count of MPs on each filter. We estimate flux from the atmosphere to terrestrial ecosystems, create a back trajectory model, and conduct statistical analysis to identify the potential sources of MPs. In this study, a distribution of MPs is shown for the first time in the Cascade and Sierra Mountain ranges, a major water supply source for communities in these two

states. Sites of various proximity to anthropogenic activity have relative MP counts ranging from approximately 20-300 MP per liter of snow meltwater. Consistent concentrations within a site, field blanks, and positive and negative controls further show that this method is replicable and robust. The future of this workflow lies in its application to measure MP presence and flux in any environmental matrix at a high throughput scale. We envision researchers will be able to expand their sampling within sites and increase the number of study sites with this automated MP detection method, and ultimately having stronger evidence of MP transport rates.

### **3.10.P-Tu211 Multifaceted Effects of Microfibers on Soil-Plant Systems: Exploring the Response Under Different Crop Cultivations**

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Microplastics (MPs) are emerging pollutants that have been receiving global attention due to uncertainties around their occurrence, fate, environmental and human health risks. While research on the impact of MPs on aquatic systems is growing, studies investigating their influence on terrestrial systems are limited. Agricultural soil serves as a dominant sink for MPs, with microfibers being a predominant form due to the application of organic amendments. Despite their ubiquity, the transport mechanisms of these particles and their effects on soil-plant systems remain largely unknown. This study focused on the impact of polyester (PES) fibers on soil quality and plant performance, selecting lettuce, Chinese cabbage, and radish as biological models. Plants were cultivated in glass jars with soil containing 100mg/kg microfibers. Soil endpoints revealed that microfibers had significant effects on soil physical properties, including a reduction in bulk density, inhibition of macro-aggregate formation, and promotion of micro-aggregate formation. These trends were consistent across all plant treatments. Additionally, microfiber addition, in conjunction with different crop cultivations, led to alterations in soil chemical properties. For instance, lettuce-growing soil pH decreased, while cabbage-growing and radish-growing soil pH increased in response to microfibers. Microfibers also slightly increased soil organic carbon content in radish-growing soil. Regarding plant endpoints, impacts varied among plant species. Lettuce germination accelerated in the presence of microfibers, while radish root length was adversely affected. Lettuce and cabbage experienced diminished chlorophyll content, while radish leaves exhibited an increase when exposed to microfibers. This study highlights the negative, positive, and neutral effects of microfibers on soil properties and plant growth. It also reveals that microfibers may affect organic carbon stocks in soil-plant systems. The observed outcomes are attributed to the distinctive characteristics of fibers, including their unique shape, surface area, and flexibility, which may interact with soil particles and crops. Given the widespread distribution and accumulation of microfibers in agricultural soil, this study provides crucial insights for ecotoxicological assessments related to soil and terrestrial higher plants. It also holds implications for stakeholders in environmental pollution, food safety, and human health.

### **3.10.P-Tu212 Quantitative tracking of nanoplastics along the food chain from lettuce (*Lactuca sativa*) to snails (*Cantareus aspersus*)**

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Terrestrial systems are a significant sink for plastic contamination, including nano- and microplastics (NMPs). Several studies showed that both invertebrates and plants are able to uptake NMPs directly from their environment. Another exposure pathway for invertebrates and higher-level organisms is the transfer of NMPs up the food web via trophic transfer. To date, only limited information is available about this exposure pathway, however, concerns about trophic transfer of NMPs in the terrestrial food chain have been raised. Our study aims to examine and quantify the trophic transfer of europium doped polystyrene nanoplastics (Eu-PS; NPs) within a terrestrial food chain. The uptake of 100 nm spherical Eu-PS particles from water through the roots of the plants to the leaves and finally to garden snails (*Cantareus aspersus*) was assessed. Lettuce (*Lactuca sativa*) was cultivated in Hoagland solution spiked with different concentrations of Eu-PS (15, 150 and 1500 µg/L) for three weeks. Then, lettuce shoots were used as food for snails for 19 days at a rate of 1 g of shoots per day. The Eu-PS primarily accumulated in the roots for all treatments, with a limited transfer to the shoots (only quantifiable in the highest treatment; translocation factor: TF<1). No detectable levels of Eu-PS were found in the snails' digestive gland; however, the Eu-PS particles were detected in their feces (trophic transfer factor: TFF>1). In addition, only limited effects were observed on lettuce biomass by the NPs treatments. No effects of the Eu-PS particles on snails were observed, with the exception of a consistent decrease in the snails' shell diameter. Overall, our research illustrates that NPs can be absorbed by plants through their roots, subsequently transported to the shoots. However, we did not find detectable trophic transfer into snail tissues, but direct elimination through their feces. Thus, our findings show limited transfer of NPs from plants to snails at a higher trophic level. This provides important insight into the potential within the human food basket. Nonetheless, NPs did cause effects on lettuce and snails, indicating a potential health risk to consumers posed by NPs which can accumulate in crops.

### **3.10.P-Tu213 Species-dependent responses of crop plants to polystyrene microplastics**

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Only recently there has been a strong focus on the impacts of microplastics (MPs) on terrestrial crop plants. Nonetheless, the large difference in experimental design between studies makes comparison of effect size and even the direction of the impacts among species difficult. This study examines and compares the effects of MPs on two monocotyledonous (barley, *Hordeum vulgare* and wheat, *Triticum aestivum*), and two dicotyledonous (carrot, *Daucus carota* and lettuce, *Lactuca sativa*) plant species through two complimentary experiments. First, we investigated the effects of low, medium, and high ( $10^3$ ,  $10^5$ ,  $10^7$  particles/mL) concentrations of 500 nm polystyrene microplastics (PS-MPs) on seed germination and early development. We found species-dependent effects on the early development, with MPs only significantly affecting lettuce and carrot. When acutely exposed during germination, PS-MPs significantly delayed the germination of lettuce by 24%, as well as promoted the shoot growth of carrot by 71% and decreased its biomass by 26%. No effect was recorded on monocot species. Secondly, we performed a chronic (21 d) hydroponic experiment on lettuce and wheat. We observed that PS-MPs significantly reduced the shoot growth of lettuce by up to 35% and increased its biomass by up to 64%, while no record was reported on wheat. In addition, stress level indicators and defence mechanisms were significantly up-regulated in both lettuce and wheat seedlings. Overall, this study shows that PS-MPs affect plant development: impacts were recorded on both germination and growth for dicots, and responses identified by biochemical markers of stress were increased in both lettuce and wheat. This highlights species-dependent effects as all crops were grown under identical conditions. Some responses, be it during short- or long-term exposure, were observed for all tested species. Although a variety of responses were observed for the apical endpoints, the biochemical markers showed a similar trend for all plants species. Biochemical markers indicate that both monocots and dicots were stressed due to the exposure to MPs, which may have further consequences on growth, functioning and yield. To conclude, research on the differences of crop specific impacts of MPs continues to be of great importance. In addition, testing under more environmentally realistic conditions, including several stressors, is necessary to use these results to make realistic interpretations for field scenarios.

### **3.10.P-Tu214 Disentangling Microplastics Effects on Oxygen Diffusion, Microbial Activity and Greenhouse Gas Emissions in Soil**

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Understanding the responses of soil ecosystems to the presence of microplastics (MPs) becomes important as multiple stressors can act together to impact this environmental compartment. MPs impact soil structure, nutrient cycling, and greenhouse gas (GHG) emissions. However, the mechanisms underpinning the direction and magnitude of MPs effects on these soil properties are uncertain, mainly due to the lack of knowledge of how the presence of MPs drives changes in soil structure and the subsequent linkages between soil structure and microbial activity. Here, we hypothesized that the presence of MPs affects soil structure, affecting pore connectivity, leading to higher or lower O<sub>2</sub> availability and consequently higher or lower soil respiration. Furthermore, we anticipated that the magnitude or direction of the effects would be dependent on soil texture and the shape of MPs. We spiked PET MPs fibers (500 µm length) and fragments (125 – 250 µm) into custom built rhizotrons (5 x 5 x 1 cm) filled with either clay or sandy loam soils (contrasting textures) with a MPs treatment of 1 w/w%. First, we determined the differences in pore connectivity in air dried soil using the rate of O<sub>2</sub> diffusion as a proxy. Next, we determined the O<sub>2</sub> concentration in the soil in optimal conditions for respiration (50% water holding capacity and addition of 20 mg g soil<sup>-1</sup> of glucose). O<sub>2</sub> diffusivity and O<sub>2</sub> concentration were determined by mapping O<sub>2</sub> concentrations using optode imaging, over the course of 48 hours. Fluxes of CO<sub>2</sub> were measured under the same conditions by placing replicate set-ups in a Tedlar bag and collecting gas samples for estimates of CO<sub>2</sub> concentration using gas chromatography. O<sub>2</sub> diffusion was faster in the sandy loam compared to the clay soil. The addition of MPs reduced O<sub>2</sub> diffusion rate in the sandy soil but increased it in the clay soil. In optimal conditions for soil respiration, the treatments showed the same trend for O<sub>2</sub> concentrations, suggesting that O<sub>2</sub> concentration in the soil matrix is likely to be regulated by pore connectivity opposed to respiration. Collectively, we show the impacts of MPs addition to soil and their impacts on the linkages between soil structure, microbial activity and GHG emissions. This study can serve as a baseline for understanding the important impacts of MPs have on soil functioning, which is relevant as plastics are increasingly used directly in agriculture and consequently have direct releases to terrestrial ecosystems.

### **3.10.P-Tu215 Comparing Approaches to Terrestrial Ecotoxicity Studies for Micro- and Nanoplastic Particles and Engineered Nanomaterials: A SWOT Analysis Approach**

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A rising interest in understanding the impact of micro- and nanoplastic particles (MNP) on ecosystems has led to an increased number of research and review articles. Initially focused on marine ecosystems, recently, interest in MNP ecotoxicological effects on terrestrial ecosystems has gained momentum. However, concerns raised around the reliability and relevance of studies on the ecotoxicological effects of MNPs highlight issues with the reporting of MNP properties and a lack of environmental representativeness [1,2]. To advance understanding of the terrestrial ecotoxicity of MNPs, it is imperative that these issues are identified and addressed.

The study of engineered nanomaterials (ENMs) ecotoxicity has parity with MNPs. Both are ubiquitous in the environment and, from a risk assessment perspective, represent non-traditional materials for which observed biological effects may be influenced by a variety of parameters (e.g. size, shape and composition). However, the study of ENM ecotoxicity is more established and, thus, by examining the differences between ENM and MNP terrestrial effects studies, we aim to identify opportunities to enhance the conduct of MNP studies.

As part of the Holistic Environmental Risk Assessment for Microplastics in the Terrestrial Environment (HERA-MP) project, a review of recent (published between January 2020 and June 2023) terrestrial effects studies for both ENMs and MNPs was performed, from which a comparative strengths, weaknesses, opportunities and threats (SWOT) analysis was conducted to facilitate a comprehensive comparison.

Both MNP and ENM studies exhibited robust experimental design and thorough reporting of basic experimental parameters. Weaknesses included poor harmonisation, limited environmental relevance, and exposure assessment. Based on ENM studies, opportunities to improve MNP studies included additional particle characterisation parameters (e.g. surface properties) and endpoints (e.g. reactive oxygen species), as well as promoting harmonisation and data sharing opportunities. Threats were associated with limited knowledge of effects mechanisms, MNP detection in soil, data transparency and resource constraints. These findings will inform future research and risk assessment of MNPs by contributing to the development of a robust risk assessment framework for MNPs in terrestrial ecosystems.

[1] de Ruijter *et al.* Environ.Sci.Technol. 54(19), 11692 (2020).

[2] Mehinto *et al.* Microplast.Nanoplast. 2(1), 17 (2022).

### **3.10.P-Tu216 Effects of microplastics pollution to soil fungi and fungi feeding springtails**

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Despite research on impacts of nano- or microplastics in terrestrial ecosystems has been increased, we know relatively little about response of springtails. The aim of this study is to investigate effects of microplastics pollution to soil fungal community and fungi feeding springtails. Test soils were amended with high-density polyethylene microplastics. Soil fungal community was analyzed and multigenerational effects on springtail *Folsomia candida* were evaluated to F2 generation. After one month of exposure period, soil fungal groups excreting toxins or entomopathogenic fungi were increased. Springtails at F2 generation were significantly affected in the contaminated soil by microplastics after three months of exposure period. As a result, we observed a correlation between MPs pollution, soil fungi, and fungi feeding springtail. These results indicated that soil health can be negatively influenced by microplastic pollution. *Acknowledgement-This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2021R1C1C2012628).*

### **3.10.P-Tu217 Polystyrene Nanoplastic (NPs) and Sulfamethoxazole: a Multidisciplinary Approach to Assess the Impact of a Contaminant Mixture on Lactuca Sativa and Rhizosphere Microorganisms**

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Plastics have significantly improved humans quality of life. However, owing to their continuous use in various daily activities, the persistence and inadequate collection and disposal of larger plastic debris, are found in environment in huge quantities. Micro (MPs between 1 to 5,000 µm) and Nano plastics (NPs, <1 µm) cause particularly concern because they can be accumulated in food chains and can represent potential adsorption carriers of organic pollutants (e.g. antibiotics, antibiotic resistance genes), increasing their environmental spread. NPs, due to their high surface area and penetration properties, can be more harmful for biota; moreover, their possible interactions with microcontaminants and in particular antibiotics in soil ecosystem are still unclear. Among others, polystyrene NPs are considered a widespread emerging contaminant arising from agricultural soil management. In this context, the aim of the work was to investigate the effects of polystyrene NPs and sulphamethoxazole (SMX), a sulfonamide antibiotic which may be adsorbed by the NPs, on *Lactuca sativa* L. plants and its associated rhizosphere microbial community. For this purpose, lettuce plants were grown on soil spiked with 20 mg/L of NPs, 20 mg/L of SMX and a mix of them (MIX, 20 mg/L NPs + 20 mg/L SMX). Soil microbial community was evaluated in terms of microbial abundance and activity and *L. sativa* plant in terms of chlorophylls and malondialdehyde content. Moreover, antibiotic concentrations (HPLC-MS/MS), resistance genes for sulfonamides (*sul1*, *sul2*) and the *intI1* mobile genetic elements were determined in soil and leaf phyllosphere at different experimental times (0, 3 and 28 days). Physical and physiological plant parameters were affected by the treatments. Microbial abundance was negatively influenced by the NPs and SMX co-presence.

### **3.10.P-Tu218 Two-generation Full life Cycle Tests with Mealworms Tenebrio molitor: Effects of Agricultural Microplastics**

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Agriculture is considered one of the most important inputs of microplastics (MPs) in soil. Most of the studies so far tested the effects of MPs during one generation after relatively short exposure times (up to 3-4 weeks), but few are available for longer exposure durations and multiple generations. With this purpose, we have investigated the effects of agricultural MPs on two generations of mealworms *Tenebrio molitor* investigating the whole life-cycle. We tested MPs obtained from two mulching films commonly used in agriculture: conventional non-degradable fossil-based (low-density polyethylene LLDPE;  $36 \pm 12 \mu\text{m}$ ; numeric distribution) and biodegradable fossil-based (poly(butylene adipate co- terephthalate; starch PBAT;  $81 \pm 38 \mu\text{m}$ ; numeric distribution). Mealworms were fed with wheat bran at 0.005, 0.05, 0.1, 0.5, 1, and 5% MPs (w/w dry weight). The first generation was exposed for 12 weeks at 20 °C. During the experiments, adults were collected and transferred to new pots with corresponding concentrations of MPs. The larvae that emerged from these adults were then synchronized for size (60-90 mg) and exposed again until the completion of the second life-cycle which was faster (9 weeks). We followed the metamorphosis of organisms (larvae->pupae->adults), larval growth, adult and pupae final emergence success, moult and mortality.

Overall tested MPs did not induce effects on the survival of mealworms. The final emergence of pupae and adults was decreased only in the case of 5% PBAT (1. generation). However, changes in moult and growth of larvae were observed in the case of both types of MP (LLDPE and PBAT) as well as in both generations. The types of responses were not the same for all types of exposures. Namely, while a trend of decreased growth was observed in the case of 5% LLDPE (1. generation), it was increased in the second generation (1% and 5%). In the case of PBAT, no effect on growth was observed in the first generation, while a trend of growth was observed at all test concentrations in the second generation with the most significant changes at 5%. Changes in moult were not as clear. Moult was decreased at 5% LLDPE (1.generation), increased at 5% PBAT (1.generation) and decreased at 5% PBAT (2. generation).

In conclusion, our results show that both types of MPs; conventional non-degradable fossil-based and biodegradable fossil-based, induce alterations in mealworm development but these are the most evident at the highest test concentration (5%)

### **3.10.P-Tu219 Lessons learned from research with model polystyrene nanoplastics in different environmental approaches**

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There is a lack of knowledge about the fate and impact of microplastics and nanoplastics (MNPs), as well as their potential uptake and impact on plants and microorganisms. It is difficult to detect polymers in soils, which explains the knowledge gaps, and the need of model particles. The presented research can be categorized into three cornerstones: First (I), commercial polystyrene particles (PS-P<sub>c</sub>, 1000 nm) were applied to quartz sand columns. Second (II), polystyrene particles (PS-P, 343 nm) and palladium (Pd) nanoparticle-doped polystyrene particles (PS-Pd-PS-P, 442 nm) were synthesized, characterized, and applied to agricultural soils for a toxicity test (Cambisol, Podzol). Third (III), polystyrene particles with a core of silver (Ag) nanoparticles (Ag-PS-P, 262 nm) were synthesized, characterized, and applied to a model wastewater treatment plant (MWTP).

(I) UV-Vis spectroscopy was used to determine the column breakthrough of the PS-P<sub>c</sub>. Adsorption of dissolved organic matter facilitated the transport of PS-P<sub>c</sub> by reducing their surface hydrophobicity. Hence, mobility of MNPs in soils seems very likely.

(II) Different techniques, such as inductively coupled plasma-mass spectrometry (ICP-MS), pyrolysis-gas chromatography-mass spectrometry (Pyr-GC-MS), and scanning electron microscopy (SEM), were used to characterize the PS-Pd-PS-P and the PS-P in dispersions, soils and plants. The spiked soils were applied to a plant toxicity test with oat. The applied particle contents could be recovered from both soils by ICP-MS (Pd, 89% - 99%) and Pyr-GC-MS (PS, 73% - 120%). Moreover, non-aggregated particles in soils and on oat roots were visualized through SEM. The ratio obtained for the Pd contents in oat roots to that in the soils (2.2 - 2.7) implied that particles accumulated on the root surface or in the roots. No Pd was detected in the oat shoots, which indicated that no translocation occurred from the roots to the shoots. Despite the particle accumulation at or in the roots, no clear effects on plant growth were observed.

(III) High amounts of the applied Ag-PS-P were retained in the sewage sludge during wastewater treatment in the MWTP. Therefore, sewage sludge applications to soils should also be considered as a source of MNPs for the terrestrial environment.

In summary, the model PS-P, PS-Pd-PS-P, and Ag-PS-P greatly benefit the development and validation of methods to investigate MNPs in complex environmental matrices.

### **3.10.P-Tu220 Do We Have the Ingredients for Soil Microplastic Policies based on Environmental Risk Assessment?**

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Recently, it has been demonstrated that microplastics are ubiquitous in the environment, including in soils, and that they pose a hazard to organisms. However, risks of microplastics remain uncertain, especially for soil ecosystems. Without scientific consensus on the actual risk and its specific drivers (e.g. the polymers and/or applications that have the greatest contribution to the risk), policy makers are limited in the options for policy actions they can take. Hence, they may have to rely on generic precautionary regulation and policy to reduce the uncertain impact of plastic pollution. In part, this uncertainty relates to the limitations with respect to the relevance and reliability of available scientific data. Furthermore, the uncertainty relates to the extent in which risk assessment approaches can deal with the complexity of microplastics pollution, i.e., the diversity of particles, physical, chemical and microbiological effects. Thus far, several scientists have proposed approaches to environmental risk assessment of microplastics and used these to estimate the risks to the environment, including terrestrial ecosystems. Pragmatic choices have been made to deal with uncertainties and the complexity of microplastics pollution. Overall, state-of-the-art risk assessments have suggested that risks to the environment cannot be excluded. The question remains whether the right ingredients for a scientifically sound risk assessment of microplastics are available that can feed policy making. And if not, which ingredients are missing or which need further development or testing? In this presentation, currently available approaches to risk assessment of microplastics in soils are presented and compared. These include novel approaches specifically designed for microplastics, as well as existing approaches for conventional substances or materials. Information needs of Dutch policy makers collected in several workshops held in the Netherlands will be presented. With this presentation and together with the audience, we strive to collect input on the current state-of-the-art of environment risk assessment of microplastics. Specifically, we want to explore future directions of research and the current scientific acceptance of available approaches and their role in the development of microplastics policy for soils. This should help ensure that science better meets policy needs, allowing policymakers to make evidence-based decisions.

### **3.10.P-Tu221 Assessment of Microplastic Emissions from Artificial Turf Pitches**

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Following the recommendation of the European Chemicals Agency, on 25 September 2023 the European Commission passed a comprehensive new regulation to reduce emissions of microplastics (MP) into the environment, which includes the sale and use of intentionally added MP. This also applies to the application of synthetic rubber granulate infill in artificial turf systems and will ultimately have an impact on recreational sports. In Germany, rubber granulate made of ethylene-propylene-diene-monomer rubber (EPDM) is currently predominantly used. So far, there is no sufficient database for estimating MP emissions from artificial turf pitches into the environment and thus their relevance as a source of MP pollution. This topic is controversially discussed due to the complexity of sampling and analytics. To close this research gap, this project has the goal to determine mass balances for the emissions of MP from artificial turf pitches to allow an estimation of the amount of MP released per turf pitch and year.

Within this study, MP emissions of three artificial turf scenarios at different ageing states (unaged, artificially and real-time aged) are compared: the past (old turf: fossil based, synthetic infill), present (1. most commonly installed in Germany: fossil based, EPDM infill; 2. turf with bio-based grass fibres and EPDM-Infill with hemp), and the future (turf with recycled grass fibres, no synthetic infill). To simulate the outdoor weathering during the lifespan of approx. 15 years, new artificial turf and EPDM granulate were accelerated aged by UV weathering and mechanical stress. Potential MP emissions into surface and groundwater are simulated by lysimeter and shake experiments. MP mass contents are subsequently determined by Thermal Extraction Desorption Gas Chromatography/Mass Spectrometry. Using special microfilter crucibles allows the estimation of the particle sizes of the emitted MP, which is a fundamental requirement for an assessment of potential health hazards for humans.

First results of shake experiments of unaged turf components to determine maximum MP contents potentially emittable into surface waters (5 - 50 µm) showed that the highest MP mass contents of 1,761.5 - 5,784.2 µg/g were determined for EPDM granulate. For the recycled artificial turf, total MP contents of 7.1 - 18.3 µg/g were determined: 1.3 - 4.1 µg/g from PE grass fibres, 0.4 - 0.5 µg/g from PP backing, 0.0 - 5.9 µg/g from PET winding yarn and 5.4 - 7.8 µg/g from PU adhesive.

### **3.10.P-Tu222 Impacts of Conventional and Biodegradable Microplastics on Plant-Soil Systems**

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Over the last few years, there has been an increasing awareness of microplastic (MP) pollution worldwide. Previous studies have confirmed their ubiquitous presence in all environments, including agricultural soils, where plastic mulch represents a major source of MPs due to incomplete recovery after each cropping season. However, understanding the potential impacts of these anthropogenic materials on soil and plant health remains limited. In this context, to assess the long-term impact of different types of MPs (conventional and biodegradable) on the growth of cotton plants and on the functioning and biodiversity of soil and the associated-plant microbiome, a cycling plant growth experiment using cotton has been conducted. The process of seed sowing, plant growth, and harvest has been repeated for a total of four cycles. Several parameters associated with plant development including indicators of plant growth and foliar stress, and community composition and metabolic activity of soil microbiota were assessed over time. The results of the plant bioassay indicate a temporary impact of biodegradable MPs on plant development. Specifically, during the first cultivation cycle, the addition of biodegradable microplastics to the soil led to reduced plant growth compared to other treatments, as evidenced by decreased values in plant height, stem diameter, aerial

fresh weight, and the number of flowers. However, in subsequent cultivation cycles, none of the evaluated parameters related to plant development showed a significant difference. Furthermore, additional data regarding potential changes in the microbial community, both in terms of their structure and physiological profile, have been collected and still need to be statistically evaluated. These findings may contribute to a better understanding of the impacts of these pollutants on the soil microbiota and, consequently, the observed effects on plant development. Moreover, assessments over time, as outlined in this project, will also provide valuable information on the temporal dynamics of the soil and plant-associated microbiome in response to MP pollution, which have not yet been fully described. Understanding the impacts of MPs on the environment may contribute to further policy formulation and recommendations aimed at addressing plastic pollution on a global scale, as well as the development of new alternatives to plastic mulch using technologies involving non-toxic materials.

### 3.10.P-Tu223 Effects of environmentally relevant mixtures of microplastics on terrestrial organisms

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Although attention has mainly been given to plastic pollution in aquatic ecosystems, soils may also act as a sink of microplastics. Little is known, however, about their fate and effects in soil. The main objective of this study was to evaluate the effects of a realistic mixture of microplastics (MPs) present in terrestrial ecosystems on two soil ecotoxicological model organisms, the earthworm *Eisenia andrei* and the springtail *Folsomia candida*. For this, high density polyethylene (HDPE) and polypropylene (PP) fragments were generated by cryomilling recycled plastic pellets, and polyester (PES) fibers were generated cutting and grinding a blanket, with a final particle size range between 36 and 3887  $\mu\text{m}$ . Chronic tests following toxicity test guidelines 222 and 232 from the Organisation for Economic Co-operation and Development (OECD) were performed for earthworms and springtails, respectively, using LUFA 2.2 natural standard soil. The organisms were exposed to the mixture of 55% HDPE, 42% PES, 3% PP in mass at test concentrations of 0, 0.64, 1.6, 4 and 10 g/kg dry soil in the test with *E. andrei*, and 0, 0.64, 1.6, 4, 10, 25, 50 g/kg dry soil in the test with *F. candida*. Survival (number of alive adults) and reproduction (number of juveniles produced) were assessed for both species and growth for *E. andrei* to determine No-Observed Effect Concentrations (NOECs). MP ingestion was determined in both species at concentrations of 0.64 and 4 g/kg, and at 50 g/kg in *F. candida*. In addition to assessing the number of ingested particles, size of ingested particles was compared to the initial size distribution. Egestion was also analyzed at the same concentrations in *E. andrei* after 24 hours of depuration. First results showed that *E. andrei* was not affected by the exposure concentrations, while *F. candida* showed a significant and dose-related reduction in the number of juveniles with a NOEC of 4 g/kg dry soil. However, MP ingestion by *F. candida* was very low (0.75 particles per individual at the highest concentration), and only observed for HDPE fragments. This suggests that MP shape can influence ingestion; however, effects may also be caused by other mechanisms, such as changes in soil properties, direct dermal effects of the MPs, or the presence of residual additives.

### 3.10.P-Tu224 Quantification of Nanoplastic Uptake and Distribution in the Root, Stem and Leaves of the Edible Herb *Lepidium sativum*

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Reports of micro and nanoplastics in agricultural settings around the world and their potentially harmful effects on plant and agricultural health, demand an immediate investigation of this issue. Research regarding the uptake and bioaccumulation of micro and nanoplastics in crops is still at its infancy and the trends and patterns underlying this phenomenon need to be explored. Although few earlier studies have been useful in examining the qualitative aspect of nanoplastic (NP) accumulation in plants, quantifying it is still a difficult task. Finding a solution to this problem could aid in our understanding of this phenomenon and allow us to investigate the distribution of NPs in plant tissues. In this work, we confirm the uptake and bioaccumulation of 100 nm polystyrene nanoplastics by the roots of the edible herb *Lepidium sativum* at exposure concentrations of 10  $\mu\text{g/L}$ , 100  $\mu\text{g/L}$ , 1 mg/L, 50 mg/L and 100 mg/L, and their effective translocation to the stem and leaves of the plant. Nanoplastic presence was confirmed in the root tips, root surface, stele, lateral roots, root hairs, stem vascular bundles, leaf veins and mesophyll and leaf epidermis including stomatal sites. Here, we also propose a novel approach for the quantification of accumulated NPs which utilizes confocal laser scanning fluorescence microscopy coupled with image analysis. Quantification results show that majority of the particles were retained in the root [ranging from 0.023/mm<sup>3</sup> at 10  $\mu\text{g/L}$  to 3.936/mm<sup>3</sup> at 100 mg/L exposure concentration]; while accumulation in the stem [0.020/mm<sup>3</sup> at 10  $\mu\text{g/L}$  to 0.683/mm<sup>3</sup> at 100 mg/L] and leaves [0.003/mm<sup>3</sup> at 10  $\mu\text{g/L}$  to 0.683/mm<sup>3</sup> at 100 mg/L] was only about 18.04% and 13.04% of the median value in roots respectively. Furthermore, accumulation in plant tissues was characterised by aggregation in the intercellular spaces and heterogeneous distribution. Apoplastic pathway seemed to be the mode of NP translocation. The proposed methodology was successfully able to quantify NPs accumulated in different plant parts with a resolution of unit mm<sup>3</sup> and a future scope for inter-sample and intra-sample comparisons. Additionally, there was a reduction of about 38.89 % in the germination rate, 55 % in plant fresh weight, as well as in root weight (> 80 %), root length (> 60 %), shoot weight (51 to 78 %) and number of lateral roots (> 28 %) at exposure concentrations of 50 mg/L and 100 mg/L. However, lower exposure concentrations did not affect plant health significantly.

### 3.10.P-Tu225 Parking Lots as a Source of Microplastics in Urban Environments: Site Characterization and Interaction With Soil Microarthropods

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Microplastics (MP, size < 5 mm) are ubiquitous in terrestrial ecosystems, being found both in soils exposed to different anthropogenic activities and in remote areas, as a result of long-range atmospheric transport and deposition. The widespread occurrence of MP in soils is as source of concern as they may impair soil health and functions as well as significantly affect soil organisms throughout their life cycle. Notwithstanding, records of local sources of MP in urban environments are still limited and urge further research on MP monitoring in order to adopt measures to preserve soil biodiversity in our cities.

This study aimed to assess: (i) the level of MP contamination in topsoil from urban parking lots in the Emilia-Romagna region (Italy); (ii) the interaction between soil microarthropods and MP within the contaminated sites; (iii) the effects of parking-related MP on the model organism *Folsomia candida* (Collembola).

Topsoil samples and microarthropods were collected in urban parking lots characterised by parking plastic grids or concrete grid pavers, in comparison. MP were extracted from soils and biota using an environmentally friendly protocol based on canola oil floatation (for soil samples) and oxidative treatment (for both types of samples). MP were then isolated on glass-fibre filters and analysed using an optical digital microscope, which allows for a semi-automatic count and size measure of the MPs. Sub-samples were further analysed by Raman microscopy to determine MP prevalent polymers, used for the ecotoxicity testing with *F. candida*.

First results showed high MP levels in the urban parking lots characterised by weathered green plastic grids. For filter portions of 10 mm<sup>2</sup>, about 1000 micro-sized green fragments made of high-density polyethylene (HDPE) were found, with a wide size distribution ranging from 20 to 830 µm (average MP size of 160 ±130 µm). These MP were further searched within specimens of soil microarthropods collected from the field to determine the presence of MP traces in their digestive tracts. Furthermore, the preliminary effects of parking-related MP (100-1000 mg/kg) on springtails (*F. candida*) mortality and behaviour were evaluated.

This study allows to understand the risk of MP to urban soil biodiversity. Our findings underline how choices in urban design can determine important sources of MP contamination. Sustainable-oriented urban planning may prevent further human-caused disturbances to urban soil biodiversity.

### 3.10.P-Tu226 Effects of conventional and biodegradable mulching film microplastics on the earthworm *Eisenia andrei*

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Microplastic contamination of soils has become a critical environmental concern, carrying potential repercussions for terrestrial ecosystems. Earthworms, being bioindicators of soil health, offer insights into broader ecological impacts through their responses. Understanding these effects is crucial for predicting and managing the consequences of microplastic contamination in terrestrial environments.

The objectives of this study were to assess the ecotoxicological effects of conventional and biodegradable microplastics on the earthworm *Eisenia andrei*, focusing on reproduction, growth, and oxidative stress. By comparing the effects of conventional polyethylene (PE) and biodegradable polybutylene adipate terephthalate (PBAT) based microplastics, the experiment addresses potential differences in their impact on earthworms.

The experimental setup included a standard eight-week earthworm reproduction test, wherein adult earthworms were exposed to seven different microplastic concentrations (0%, 0.005%, 0.05%, 0.1%, 0.5%, 1%, 5%), ranging from environmentally relevant to high concentrations. After the first four weeks, adults were removed, counted, weighed, and transferred into biomarker assays. The experiment continued with juveniles for an additional four weeks, with the number of juveniles calculated. Additionally, oxidative stress, a critical indicator of an organism's physiological well-being, was evaluated by analyzing five different enzymatic antioxidants and lipid peroxidation levels in tissues of adult earthworm. These parameters contribute to forming a Biomarker Index, providing a comprehensive overview of the antioxidant system's functionality across various microplastic treatments. Furthermore, the soil water-holding capacity and pH were measured.

We anticipate identifying differences in response patterns between conventional PE and biodegradable PBAT microplastics, reflecting the distinct ecological implications associated with these materials.

Filling the knowledge gap in this field is crucial, and this experiment will provide new information on the interactions between earthworms and microplastics in terrestrial environments. The study is part of the PAPPILLONS, funded under Horizon2020.

### 3.10.P-Tu227 Tracking Polyethylene Degradation in Soil Using a <sup>13</sup>C-labelling Approach

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Plastic waste is pervasive in agricultural soil, with polyethylene (PE) being the largest contributor, known for its recalcitrance. Despite this, polyethylene degrades into micro- and nano-plastics in soil. These can affect soil properties and plant health, posing risks to ecosystem services. However, quantitative bacterial mineralization rates of polyethylene, as well as the fate of polymer-derived C in different soil compartments (incorporation into microorganisms, soil structure, etc) are not known. Traditional techniques, such as spectroscopic and imaging techniques, weight loss studies, and microbial respiration lack precision in quantifying PE degradation rates and confirming microbial involvement. Employing <sup>13</sup>C-labelled PE is a promising approach, offering precise quantification of mineralization rates and the ability to track polymer-derived carbon assimilation by microorganisms. This can be done by sequencing of the <sup>13</sup>C-containing DNA (identifying plastic degraders) and through analysis by nanoscale secondary ion mass spectrometry (NanoSIMS, tracking the polymer-derived C in soil).

This study incubates <sup>13</sup>C-labelled PE in a soil microcosm for 2 years. CO<sub>2</sub> formed through microbial mineralisation is trapped in NaOH and quantified via total inorganic carbon concentrations and  $\delta^{13}\text{C}$  values are determined via elemental analysis isotope ratio mass spectrometry (EA-IRMS) every 4 weeks. Soil samples are collected at 1, 6, 12, and 24 months to identify plastic-degrading microbes, and ploughing/harvest is simulated through addition of organic matter to the soil every 6 months. Initial results show 0.073 mol% of C in PE degraded after 4 months, with a decreasing monthly degradation rate (0.027, 0.020, 0.016, and 0.009 mol% for month 1, 2, 3, and 4). By May 2024, results for the first 9 months of the experiment as well as isolation of microbes responsible for degradation will be available.

The <sup>13</sup>C-labelling approach allows for quantification of PE mineralization at very low rates and short timescales and can provide unequivocal proof of biodegradation by microorganisms. Further, will be able to map incorporation of polymer – derived C into soil and microorganisms via NanoSIMS and DNA sequencing. Our study offers an important contribution to understanding the environmental fate of polyethylene, allowing risk assessment to be carried out and addressing pressing concerns related to soil health, crop productivity, and food security.

### 3.10.P-Tu228 Toxicity assessment of metal mixtures on *E. crypticus*: effect of Polyethylene microplastic

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The ubiquitous presence of plastics, notably Polypropylene (PP), polyethylene (PE), and polystyrene (PS), coupled with the accumulation of heavy metals including Cadmium (Cd), Copper (Cu), Lead (Pb), Nickel (Ni), Zinc (Zn), and Chromium (Cr) in natural ecosystems, raises critical concerns regarding environmental health. Both microplastics and heavy metals pose significant threats as they infiltrate soil, water systems, and ecosystems, impacting diverse organisms and ecological balance. This research aims to elucidate the complex interactions between microplastics and heavy metals in soil environments, focusing on their combined impact on *E. Crypticus* nematode a crucial component of soil ecosystems. While individual toxic effects of these pollutants are well-documented, their combined impact remains a critical knowledge gap, especially concerning multiple metal mixtures. This study pioneers an investigation into the synergistic, antagonistic, or additive effects of PE microplastics in the presence of six heavy metals, examining their influence on the reproductive toxicity of *E. crypticus*. The experimental design includes testing binary and six-metal mixtures against regulatory standards and metal NOEC values. By employing concentration addition and independent action models, the study evaluates the suitability of current regulatory guidelines in estimating mixture toxicity and elucidates discrepancies between regulatory limits and scientific realities. Results indicate substantial alterations in metal toxicity within the presence of PE microplastics, highlighting the importance of considering individual NOEC values for accurate toxicity assessments in mixtures. The study unveils synergistic effects among metals and the role of microplastics in altering metal toxicity profiles, emphasizing the need for a more realistic assessment framework for environmental regulations. This pioneering investigation contributes significantly to understanding the combined effects of microplastics and heavy metals on soil organisms, providing essential insights for regulatory evaluations and emphasizing the necessity of incorporating NOEC values for more effective environmental regulations. The findings underscore the urgency of further research into the fate of metals and microplastics in soil matrices, essential for developing predictive models and refining environmental risk assessments.

### 3.10.P-Tu229 Earthworm (*Eisenia andrei*)-Mediated Degradation of Commercial Compostable Bags and Potential Toxic Effects

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The availability of compostable bags has greatly increased over the past few years. Consumers generally perceive that the bags will be degraded after disposal. However, there are still some knowledge gaps regarding the potential effects on the ecosystem due to improper disposal of this type of bags. With the development of degradable polymers such as poly (butylene adipate co-terephthalate) (PBAT) or polylactic acid (PLA) and their application, one such gap is the role that the composition of compostable bags have in their degradation and potential toxic effects.

In the present study, we assessed the degradation rate of polymer films from 4 different types of commercial compostable bags (PLA/PBAT/corn starch, Bioplast (PLA)/PHA, Mater-Bi/corn starch and PBAT/potato starch) in continuous vermicomposting systems, with the earthworm species *Eisenia andrei*, using two different substrates: sewage sludge (SS) from wastewater treatment plant (WWTP) and spent coffee grounds (SCG). This was complemented with the assessment of the biological response of *E. andrei* (survival and reproduction) to microplastics obtained from fragments of the plastics (< 2000 µm) and the seeding emergence in common garden cress (*Lepidium sativum* L.) exposed to micronized plastic (<250 µm) and the respective leachate, following OECD and ISO guidelines respectively.

The degradation rate of the plastic samples differed significantly depending to the type of plastic used in the vermicomposting system, with Bioplast (PLA)/PHA being less degraded, while the addition of starch, e.g., from potato, potentiated the degradation of polymers. These findings suggest the degradation process is more dependent on the microbial community that can colonize the different types of plastic than the earthworm activity. Regarding the biological response of the soil systems, the *L. sativum* seedling emergence was not significantly affected, however it was observed that the microplastics (MPs) of the plastic bags can affect earthworm reproduction, with the less degraded Bioplast (PLA)/PHA being the most toxic. This suggests that although compostable, some of these formulations may potentially be toxic for soil fauna.

### 3.10.P-Tu231 From Particles to Plants: Exploring Nanopolystyrene's Impact on Lettuce Growth

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In horticulture, plastics are crucial in enhancing crop yield and quality. Nonetheless, plastic contamination, notably micro- and nanoplastics, is a growing concern for the potential adverse effects on soil properties and terrestrial organisms. Plastic impact assessments commonly use particles which can contain synthesis impurities and stabilisers, necessitating their impact assessment alongside the plastics. The objectives of this research were to assess the biological response of lettuce (*Lactuca sativa*) exposed to increasing concentrations of nanopolystyrene (*n*PS) using model particles in a laboratory germination and hydroponic system, and to discern whether observed results could be attributed primarily to the *n*PS over synthesis impurities. Here, the synthesis, characterisation, and purification of 30nm *n*PS is described. Increasing concentrations of 0, 0.01, 0.5, 500, and 1000mg/L *n*PS, or surfactant impurities, were used in the exposure to lettuce. Thermogravimetric analysis quantified the *n*PS residual surfactant to inform surfactant controls relative to the *n*PS mixture. To assess the impacts on lettuce, germination rate, biomass, photosynthetic pigment, water uptake, and elemental analysis were measured. Purification of the crude *n*PS achieved 70% surfactant removal. After 3 days, 95% of unexposed seeds had germinated, with a statistically significantly higher germination rate than all *n*PS exposures; however, after 8 days, no significant difference was observed. The surfactant had no impact on germination. After 28 days of *n*PS exposure, all measured parameters demonstrated decreased plant growth with increasing *n*PS concentration, except for leaf moisture and photosynthetic pigment content. Surfactant only exhibited a statistically significant decrease in total leaf area and leaf fresh/dry weight at high exposures, although considerably less than with *n*PS. These findings display the importance of adequate plastic impurity controls for high concentration exposure experiments. Significant negative results on lettuce head growth and root system integrity were discernible even at low level *n*PS exposure. This poses a concern for horticultural systems, as the continued influx of plastic could progressively compromise lettuce yield and quality, harvest delays, and revenue. Insights into nanoplastic-crop impacts can inform long-term cultivation practices; current research will determine *n*PS uptake and translocation by lettuce as a potential food system input.

### 3.10.P-Tu232 Nanoplastics in Terrestrial Ecosystems: Linking Exposure to Effects Through Dose-Response Relationships

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To date, many studies have focussed on the toxicity of nanoplastics (NPs) on aquatic species, yet there is little information available about their impacts on terrestrial organisms. Moreover, studies which have exposed terrestrial organisms to NPs often could not fully assess particle ingestion due to the methodological difficulties of NPs identification and quantification. Here, we achieve NPs quantification within soil organisms with Pd-doped NPs, which can be detected via ICP-MS and can be quantified at magnitudes of orders lower concentrations than previously observed in NPs research. We exposed two important soil organisms, *Folsomia candida* and *Enchytraeus crypticus*, to such NPs in a range of 0 – 5000 mg/kg through spiked feed. Both species were fed *ad libitum* for a period of 21 days. Biological endpoints monitored in assessment of organism health included growth, reproduction, and mortality. Following the exposure period, a subset of organisms were fed a NPs-free diet to quantify particle depuration over a period of 72 hours. Our goals were to 1) quantify NPs ingestion and retention within soil organisms, and 2) investigate the dose-response relationship between NPs ingestion and organism health. While data analysis is still ongoing, we have already quantified nanoplastic ingestion within organisms above background levels at expected limits of detection, with a difference in uptake observed between *F. candida* and *E. crypticus*. We hypothesise that NPs ingestion impacts terrestrial organism health, with resultant effects on growth and reproduction. Through this work, we demonstrate the utility of Pd-doped NPs in quantifying the bioaccumulation of NPs at trace concentrations within organisms, and in enabling dose-response ecotoxicology studies in the field of nanoplastics. Furthermore, we elucidate the risks of NPs to terrestrial organisms beyond growth and reproduction. Collectively, these results on exposure and impact will contribute to the assessment of risks of NPs to terrestrial ecosystems.

### 3.10.P-Tu233 Seasonal and Population-Driven Microplastic Pollution Highlighted by Wastewater: Soil-Aquifer Treatment as a Potential Solution for Mitigation

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While the study of temporal trends is common for other contaminants of emerging concern (CECs), microplastics (MPs) have not been as extensively investigated in this context. This research aims to address this gap of knowledge by examining the seasonal variations in MPs pollution in wastewater treatment plants (WWTPs) in the coastal and tourist-heavy regions of Cambrils and Palamós, Spain. In addition a soil aquifer treatment (SAT) system, which consists of different reactive barriers irrigated with the effluent of Palamós' WWTP, has been studied as a sustainable, efficient, and innovative mitigation approach to reduce massive release of these pollutants into the environment.

Wastewater samples were collected from both WWTPs, at the three different stages of the treatment process. Autosamplers were used to collect 24-hour composite samples. Regarding the SAT system, sampling was performed by grab sampling method, following the dismantling of the reactive barriers. Subsequently, MPs were isolated from the samples performing an oxidative treatment followed by density separation. The solution is filtered through a silicon filter. Once particles are retained onto the filter, visual characterisation is performed under the microscope to evaluate the morphology, color, and size of the MPs. Afterwards filters are analysed by FTIR *imaging*, using a Nicolet™ iN™ 10 MX infrared imaging microscope.

Preliminary results revealed higher MP concentrations in effluent, with subsequent treatment stages significantly enhancing removal. The presentation will delve into polymer distribution across treatment stages, providing insights into the seasonal variation's impact on MP removal in WWTPs. SAT system barrier materials exhibited MP concentrations ranging from 60 to 236 nkg<sup>-1</sup>, showcasing high accumulation and retention efficiency. Dominant morphologies were fragments (60%) and fibers (17%), primarily white (51%) and transparent (20%). Polypropylene (PP) constituted 47%, followed by polyethylene (PE, 34%).

These results stress the need for continuous monitoring and chemical analysis of MPs in WWTPs to comprehend their source, pathways, and environmental impact, guiding effective mitigation strategies. They also underscore the link between tourism, population growth, and MPs pollution in wastewater. Effective mitigation approaches, like SAT systems, are crucial for improving wastewater treatment and safeguarding ecosystems from microplastics pollution.

### 3.10.P-Tu234 Vertical Transport of Microplastic Fragments from Mulching Films and Associated Chemical Additives in Soil Ecosystems

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Soils are an important environmental recipient of microplastic pollution. A dominant source of microplastic to soils is the degradation and fragmentation of plastic mulching films during and after use. Yet, very little is known about the environmental fate of these particles. This is important, as the fate will determine the magnitude and spatial extent of exposure – including whether soil environments represent a release pathway of contamination to other environments or if particles are instead effectively retained and could reach high concentrations following successive inputs over time. These particles may also represent a source of chemical additive to soils, the environmental fate of which is also poorly understood. This study tracked the vertical transport of microplastic fragments derived from relevant plastic mulching films and associated chemical additives within soils. This included an investigation of the influence of potentially relevant factors that may affect fate: bioturbation and soil water inputs.

The experiment was conducted in the CLIMECS (CLImatic Manipulation of ECosystem Samples) facility at Vrije Universiteit Amsterdam, which comprises 40 soil columns that simulate ecosystems – with soil, vegetation, and fauna – and are individually controlled for different environmental conditions. Two types of microplastics: fragments of conventional (LLDPE) and biodegradable (starch-PBAT blend) plastic mulching films were added to the upper layer (10 cm) of soil columns (total length: 40 cm) to represent a contaminated plough layer. Different treatments included high and environmentally relevant microplastic concentrations (0.5% LLDPE + 0.5% starch-PBAT and 0.05% LLDPE + 0.05% starch-PBAT, respectively), high and low watering regimes, and the presence and absence of earthworms. The columns were maintained for a period of three months. At the end of the experiment, each column was divided into six depth layers and the microplastic and chemical additive content was measured at each depth to assess the vertical transport of particles. The results from this study reveal important insights on the mobility of mulching film fragments within soil systems and some of the important controls on particle movement. This provides crucial context related to the exposure of soil environments to soil microplastic and chemical additive pollution derived from mulching film use.

### **3.10.P-Tu235 Adding it up: Effects of microplastics with additives and increased monomer contents to springtails (*Folsomia candida*)**

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Antioxidant additives are used in plastic materials to delay their oxidation and prevent aging. When such materials enter the environment, for instance through inappropriate disposal, these additives may leach from the polymers, particularly when they come into contact with non-polar substances or when they are taken up by organisms and translocate into fat tissues. Despite the growing evidence for negative effects of micro- and nanoplastic particles (MNPs) on various organisms, the effects of plastic-associated additives are still largely unknown. In particular, it is often unclear if observed effects of MNP are causally linked to the polymer particles themselves or if they are instead caused by the additives. Generalizations are even more difficult considering that additives and their concentrations are usually not disclosed by the manufacturers and are thus unknown. In addition to these intentionally added chemicals, the monomer contents in MNP can vary and influence the particles' toxicity.

In the presented study, we assess the impacts of polystyrene (PS) fragments with two different additives (Irganox 1010 and Irgafos 168) at two defined concentrations and PS particles with different monomer contents on the springtail *Folsomia candida*. We compare the springtails' responses for two different exposure routes, i.e. via the soil substrate and via feeding. The endpoints assessed include mortality, reproduction, and avoidance behavior. We discuss the results in the context of increased plastic accumulation particularly in soils and develop hypotheses on mechanisms that may be causally linked to the observed effects.

Keywords: soil organisms, microplastics, additives and monomers

### **3.10.P-Tu236 Plastic Mulch And Pesticides Residues Effecting The Lettuce Growth: Insights of the soil Microbiome and nutrients availability**

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In arid and semi-arid regions, the use of plastic mulch and pesticides in conventional agriculture is nearly ubiquitous. The most common plastic used for mulching is Low Density Polyethylene (LDPE). LDPE mulch needs to be removed after harvest and LDPE debris accumulates in the environment. Some plastic producers have tried to avoid plastic mulch removal and plastic debris accumulation by adding pro-oxidant additives to LDPE, so called Pro-oxidant Additive Containing (PAC) plastics or by using biodegradable polymers (BIO). However PAC plastics leave residues when ploughed into the soil and some BIO plastic don't degrade as fast as expected in the field conditions. Similarly pesticides are known also to accumulate in the soil.

Studies investigating the effect of these contaminants on the soil fertility most often consider pristine plastic or single pesticides. We investigated the effect of one year incubation of plastic debris and pesticides in soil on the growth of lettuces in south east Spain. More precisely, we tested three plastic mulch residues and 3 commercial pesticides, including a treatment with the cocktail of the 3 pesticides compared to controls for a total of 20 treatments. The plastic mulches were first exposed 3 months to the summer weather in the field, cleaned, cut, grounded into macro- and micro-plastics debris and added in 1kg soil mesocosm at a 0.1% content w/w. Pesticides were applied 3 times, every 6 months, following the prescribed application doses. The 1kg soil mesocosms were incubated 1 year and were irrigated to recreate farming conditions. After one year lettuces seedlings, *Lactuca sativa*, were planted in the incubated soil. The plant growth was assessed during the growing period (number of leaves, chlorophyll content, stomatal conduction) and at the harvest (fresh and dry biomass, leaf area, stem diameter). The soil chemical properties were analysed again. The soil bacterial and fungal communities were analysed through 16S and ITS sequencing and qPCR. In the previous SETAC conference we showed that lettuces growing in soil incubated with the BIO plastic produced a significantly lower biomass compared to the control or the soil incubated with LDPE plastic. We left the audience with two hypothesis to explain the lower biomass i) Is it due to a lesser availability of nutrients? ii) Is it due to a change in the soil microbiome? The microbiome and nutrient data is being analysed to answer this two questions.

### **3.10.P-Tu237 Linking the occurrence of microplastics and adsorbed organic pollutants in beaches from the Cantabrian Coast (Northern Spain)**

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Microplastics (MPs) are not only considered dangerous for the environment due to their petrochemical origin, but also because they have the ability to release their manufacturing additives and concentrate pollutants from the surrounding environment. In addition, their small size makes them easily dispersed in all environmental compartments (water, soil and air), increasing the risk of ingestion by living beings in affected ecosystems, being accumulated and redistributed through food chains. Thus, they are a clear route capable of increasing the distribution, bioavailability, bioaccumulation and ecotoxicity related to them. Nevertheless, the MPs potential as environmental indicators have not been yet exploited, as most of the studies are often

focused on their monitoring to identify and quantify which type of plastics are present instead of what they carry on. Thus, this work aims to both identify and quantify the MPs that are arriving to the Spanish northern coast beaches, and assess the levels of organic pollutants that are adsorbed into them. For this purpose, a bi-annual sampling campaign was performed in three significant points of the Cantabrian Coast (Bay of Biscay) to cover pristine, touristic and urban scenarios. As the MPs role as reservoirs depends on their surroundings but also on the nature of the plastic matrix, firstly, a non-destructive characterization based on hyperspectral imaging (HSI) was used to classify and quantify the MPs collected. After that, the occurrence of organic pollutants was assessed using both a target analysis and a suspect screening by gas chromatography coupled with mass spectrometry (GC-MS) using an orthogonal approach with separation in non-polar and polar stationary phases.

According to the results, 3 types of polymers were found as main components of the MPs matrices: polyethylene (PE), polypropylene (PP) and polystyrene (PS). Besides, the presence of 50 organic contaminants (PAHs, PCBs, pesticides, UV filters and fragrances) were quantified showing the highest levels for fluoranthene, cashmeran, PCB 29, tonalide, lindane and PCB 154. In addition to the targeted analysis, several additional compounds were tentatively identified by suspect screening. Thus, this research evidenced the role of MPs as vectors for transport and release of organic contaminants in aquatic environments, and the potential of their use as indicators of the environmental risk of the compartment studied.

### **3.10.P-Tu238 Fate of Antimony (Sb) from polyethylene terephthalate (PET) micro and nanoplastics: combined toxicity study in soil-plants**

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Polyethylene terephthalate (PET), is a thermoplastic and non-biodegradable polymer with significant commercial use and they make up a significant fraction of plastic waste and are ubiquitous in wastewater treatment sludge in the form of microplastics (MP) and nanoplastics (NP). When this sludge is applied as cheap fertilizers, soils organisms and plants are exposed to PET, but also to additives. Antimony (Sb), is an additive used in the production of PET and can be found in various amounts in the matrix of the finished product.

The release of Sb from PET into water or food under high temperature, has raised concerns in the food and beverage industry, and the migratory properties are being researched. However, such migration in the terrestrial environment has not yet been investigated. Plastics (MP & NP) and metals, including Sb, are soil pollutants of concern that are reported to affect the soil biota. Moreover, their coexistence and the impact of nanoplastics in plants mechanisms is not studied or fully understood. We therefore studied Sb migration from PET (MP & NP), Sb speciation in soils, uptake of Sb or PET by wheat or peas and the resulting effect on toxicity to these plants.

Pot experiments using an agricultural soil were set up in a controlled chamber environment where wheat and peas plants, were used as model crops. The plants were grown until full maturation and the life cycle of Sb and PET (MP & NP) in the soil-plant system was analysed. The plants were subjected to different treatments including weathered PET micro and nanoplastics, along with two inorganic species of Sb (Sb (III) & Sb (V)). The soil and plant samples were characterized for elemental concentration using ICP-MS, and direct speciation of Sb is investigated by X-ray absorption spectroscopy (XAS) at Sb K-edge. Additionally, varied concentrations is analysed in pre-experiments to understand the toxicity of these treatments in young plants. The results showed toxicity behaviour in plants respect to their height and biomass for grounded PET of 0. 25% treatment compared to control and other treatments.

The results of the work will help to know the migration effects of Sb from PET in soil, co-existence of PET and Sb, Sb speciation in soil, and in particular those up taken by plants and whether the speciation influences the effects in the concerned mechanisms. It will help to understand and control the harmful migration effects of Sb, micro and nanoplastics in soil and plant systems.

### **3.10.P-Tu239 Combining FTIR and Py-GC-MS to monitor microplastics in an agricultural watershed during dry periods**

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The last decades have shown an intensified focus on the analysis of microplastics present in the environment, with a recent interest in the continental environment. Only limited knowledge is available regarding the sources and transfer of this pollution in rivers under agricultural impact. This study aims at enhancing the understanding of microplastic transfer along an agricultural watershed.

This study evaluates the microplastic contamination level of a river in an agricultural watershed (Orgeval, 50 km<sup>2</sup>) 70 km east of Paris. Sampling took place during dry period over a month and enabled us to collect eleven samples. Samples are collected



using a plastic-free pumping system combined with in-situ filtration (UFO, particle sizes 10-300 µm) with a median value of 206 L of water filtered.

Samples underwent pre-treatment protocol to remove organic matter and the mineral matrix and were then recovered on Anodisc filters. Three procedural blanks were conducted during sample pre-treatment and showed significantly lower microplastic levels than in samples. Two analytical methods were employed: pyrolysis-gas-chromatography-mass-spectrometry (Py-GC-MS) and Fourier transform infrared microspectroscopy (µFTIR).

Concentrations varied from 111 to 2088 items/m<sup>3</sup> (median: 655 items/m<sup>3</sup>), with samples primarily comprising polyethylene (80%) diverging from a study conducted on the same site on total atmospheric fallout showed predominantly polypropylene (70%) microplastics indicates differing pollution origins.

Among the five targeted polymers with Py-GC-MS, only PP was present in levels above the detection limit. By combining the analysis results with the flow rate, mass fluxes could be calculated for PP. The mass flux values obtained by µFTIR range from 0.01 – 1.11 mg/day with a median value of 0.12 mg/day, whereas values range from 0.07 – 1.20 mg/day but with a median of 0.38 mg/day in Py-GC-MS. Both methods provide results on the same order of magnitude for the PP fluxes.

Although these results are preliminary, they provide crucial information for understanding the dynamics of microplastics within the watershed. The differences in typology between microplastics from atmospheric fallout and those from rivers suggest that the sources of atmospheric microplastics differ from those in the hydrosystem. Moreover, the combining of Py-GC-MS and µFTIR indicates the potential to use these methods for estimating PP fluxes in natural aquatic ecosystems

### **3.10.P-Tu240 Ecological Impacts of Microplastics and Other Contaminants in Urban Waste Products Used as Fertilizers in Agroecosystems.**

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Recirculation of urban organic waste as fertilizers in agriculture offers multiple benefits, i.e., reducing reliance on NPK fertilizers, enhancing soil carbon content, thus positively affecting climate and plant yield. However, waste products also contain xenobiotic residues, including microplastics, raising concerns about their impact on terrestrial ecosystems.

Based on a review of published data, the study aimed to evaluate the prevalence and effects of microplastic pollution in agricultural soils, particularly from recycled waste products like household waste and sewage sludge. A case study related to a long-term Danish field trial, CRUCIAL, where individual plots were fertilized with urban waste products or agricultural reference treatments since 2003, was included in the analysis.

The analysis, not surprisingly, revealed that urban waste products used as fertilizers contribute to microplastic pollution in soils, with significant variability in concentrations. Most effect studies were conducted at concentrations above what is environmentally realistic and focused on effects at the sub-organism or organism level. Furthermore, many studies failed to observe effects within the tested concentration range. The CRUCIAL-related experiments with soil organisms showed no negative effects on soil health due to urban waste products.

The study highlighted the necessity for improved quantification methods for microplastic pollution in soils and emphasized the need for ecologically relevant impact studies. It was concluded that current levels of plastic pollution pose a limited risk to agroecosystems based on existing data. However, this conclusion is constrained by knowledge gaps regarding higher biological impacts and ecological effects under realistic exposure conditions. Standardized analytical methods and long-term field studies could provide a more comprehensive understanding of microplastic fate and impacts in agroecosystems, aiding in a more robust risk assessment.

### **3.10.P-Tu241 Assessing the Impact of Microplastics on Soil Resilience**

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Soil ecosystems are highly dynamic self-organizing complex systems. They are spatially extensive and cover 71% of the global land surface; yet, soil resilience is largely underpinned by processes physically occurring at the micrometre scale. Even small changes occurring at this scale can introduce knock-on effects across much wider scales and there is an intricate interplay between the physical, chemical, and biological components of soil. The potential for microplastics to disrupt several of these physical, chemical, and biological components has already been documented in the scientific literature. Hence, microplastics have the potential to impact soil resilience. Addressing microplastic effects by considering the responses of individual organisms or properties in isolation is insufficient to understand the role of microplastics as both widespread habitat modifiers and direct toxicants. Instead, microplastic pollution should be interrogated within the content of soil resilience as part of a more holistic assessment of impact.

Several approaches to quantify soil resilience in response to various disturbances have been proposed in the literature. These measure different aspects, such as the characteristic return time, renewal rate, or ability to resist stress. However, many of these are compiled to specifically address extrinsic and transient factors and are not necessarily directly transferable in the case of a persistent, particulate, and heterogeneous contaminant such as microplastics. An adapted approach is required to measure the impact of microplastics on soil resilience. Here, we present the outcome of a laboratory experiment to track the evolution of microplastic effects on soils over time and use these data to propose a frame for assessing the impact of microplastic on soil resilience. The laboratory experiment measured changes in several soil physicochemical and biological properties over a period of 6 months in response to exposure to microplastic fragments derived from agricultural mulching films. This reveals how soils respond and adapt to the addition of microplastic particles, including a conventional polymer type (which persists for the duration of the experiment) and a biodegradable polymer type (which transforms over time). The new statistical frame for assessing soil microplastic pollution in the context of soil resilience represents a new holistic tool for evaluating microplastic impact in soil ecosystems.

### **3.10.P-Tu243 Evaluation Method of Ecotoxicity for Biodegradable Plastics II**

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Marine pollution caused by plastics has become a global environmental problem. The prevailing use of biodegradable plastics is one of the solution, and their development is underway around the world.

The new materials are expected to have no ecotoxicity with their high molecular weight at the point of manufacturing and shipping, although, the impact of their degraded products are concerned because they are supposed to be decomposed in the environment and there is not established method for evaluating the environmental safety of biodegradable plastics.

Currently, there is a test method for the ecotoxicity of biodegradable plastics, ISO 5430, which is proposed for assuming the oceanic degradation. Although the method is designed only for the toxicity of soluble components of the sample. So that those method is not suitable for evaluation of the biodegradable plastics with their decomposed intermediate products which should be taken into account.

Also, a wide variety of structures and properties of the biodegradable plastics makes difficulties for uniform application of any method to those samples.

Therefore, the purpose of this study is to investigate the environmental impact under more realistic conditions.

First, the biodegradable plastics are classed by hydrolysis rate, and sorted into biodegradable or not based on those hydrolyze or not. In those flow scheme, when samples are assumed biodegradable, the ecotoxicity tests will be conducted.

We tested five representative biodegradable plastics on fish, crustaceans, and luminescent bacteria using above scheme and those results will be presented.

### **3.10.P-Tu244 Assessment of Microplastic Presence Along an Atlantic Coastal Region**

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Microplastics are currently affecting numerous aquatic systems worldwide. The significant prevalence of microplastics in the environment has attracted the scientific community's interest in monitoring these contaminants across various aquatic environments and matrices, including ocean floor sediments. Implementing policies and legislation to control and manage (micro)plastic pollution is crucial for safeguarding the environment, particularly coastal areas. The development of regulations should be informed by the recognised significance and trends of this form of pollution. The true importance and potential sources of this contamination can only be comprehended through the objective characterisation of contamination levels and trends.

The present work describes the first bottom-up evaluation of the uncertainty of the quantification of microplastics in environmental sediment samples, taking into account the sampling uncertainty.

This evaluation involved the identification and quantification of systematic and random effects affecting the analysis, using different probability density functions. Poisson-Lognormal distribution was used to model the random and systematic components that affect the counting of microplastics, and continuous rectangular and *t*-Student distributions were associated with uncertainty components of the sediment sample weighting. The sampling uncertainty over the 700 km<sup>2</sup> studied oceanic area was characterised considering the contamination heterogeneity and representativeness of sampling. The distribution of the microplastics number concentration was modelled by combining the above distributions and sampling uncertainty components by the Monte Carlo method. The confidence limits that enclose the true value of the contamination were defined for a 99% probability.

The methodology developed was successfully applied to the quantification of the following types of particles in sediments: i) microplastics, regardless of their physical-chemical properties; ii) polyethylene terephthalate (PET); iii) polypropylene (PP); and iv) polyethylene (PE). This investigation unequivocally demonstrates that the contamination level in the studied area did not significantly change between 2018 and 2019, with PET microplastics being the predominant polymer type. The collected

data pertaining to the environmental area play a crucial role in establishing an objective and binding assessment of microplastic contamination significance.

### **3.10.P-Tu245 The Deterioration of Bio-based PLA Plastic Teabags Under Natural Soil Conditions and Their Effect on Earthworms**

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In response to the widespread concern about plastic pollution, countries have set targets to eliminate or make plastic packaging reusable, recyclable or compostable. This includes teabags, where manufacturers have substituted the conventional plastic content with bio-based, biodegradable polymers, such as PLA. The rate at which PLA degrades varies widely within and between industrial facilities and home composting, which can result in plastic residues being transferred to the natural environment through the application of compost. Despite widespread use, the degradation rate of PLA/PLA-blended materials in natural soil and their effects on soil biota are not well quantified. The deterioration of teabags with differing PLA:cellulose compositions, exposed to natural soil (-10cm depth) for 7-months were examined using a suite of analytical techniques. The effect of 28-days exposure to teabag discs at environmentally relevant concentrations (0.02 %, 0.04 % and 0.07 % w/w) on the survival, growth and reproduction of the soil detritivore *Eisenia fetida* was assessed (OECD 222). After 7-months, 1:3.5 PLA:cellulose blend teabags and 1:2.4 blend teabags had lost on average 82 % and 66 % of their mass respectively, while 1:0 PLA bags remained unchanged. Changes were attributed to degradation of the cellulose component of the teabag, confirmed by microscopy and FTIR spectroscopy. Earthworms exhibited different resource allocation strategies in response to the teabag compositions between treatments. The reproductive output of earthworms was significantly suppressed following exposure to  $\geq 0.04$  % w/w of the 1:0 PLA teabags, however survival and growth were not impeded. In contrast, earthworm mortality increased and egg production significantly increased following 28-day exposure to 0.07 % w/w of the 1:2.4 PLA:cellulose blend teabag. Consequently, we show that the PLA:cellulose content of teabags affects degradation and can have complex effects on the reproductive output, and thus population dynamics, of the ecologically important earthworm *E. fetida*. The results highlight the necessity to better understand the environmental fate and ecotoxicity of PLA/PLA-blended materials, often proposed as plastic alternatives.

### **3.11.A Fate and Toxicity of Metals: Recent Scientific Advancements, Challenges for (Data-Poor) Metals, and Application to Environmental Regulations**

#### **3.11.A.T-01 Effects of Biochar amendment on earthworm heavy metal bioaccumulation from e-waste contaminated soil**

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Waste Electrical and Electronic Equipment (WEEE), commonly referred to as e-waste, has emerged as a rapidly expanding waste stream and a significant environmental issue globally. The growth and reproduction of earthworms exposed to e-waste contaminated soils has been shown to be severely inhibited and this was linked to heavy metal uptake and bioaccumulation by the earthworms. Biochar is recognized for its ability to remediate various contaminants from soil, including excessive heavy metals, by reducing their bioavailability and disrupting their exposure pathway. However, effects of biochar applications on macrofauna such as earthworms have received very limited attention. The effects of 1% biochar amendment to e-waste contaminated soil on heavy metal bioaccumulation by the tropical anecic earthworm, *Alma nilotica*, was studied. We found that the concentrations of all five heavy metals studied (Cd, Cu, Ni, Pb and Zn) decreased continuously from the amended as well as unamended e-waste soil over the study duration, while increasing in the exposed earthworms. More interestingly, the earthworms bioaccumulated the metals more in the unamended e-waste soil when compared to the biochar-amended soils, despite the fact that the amended soils contained a higher metal concentration. Consequently, the bioaccumulation factors (BAFs) of all five heavy metals were 3.7 to 5.8 times higher in the unamended e-waste soil compared to the amended soil. Pb had the highest BAF, followed by Cu and Zn, which had very similar BAFs. These results confirm the potential of biochar for soil heavy metal remediation. However, it is necessary to determine if this observed lowering of heavy metal uptake and bioaccumulation will correspond to increased earthworm growth and reproduction.

#### **3.11.A.T-02 Antimony Release Upon Soil Flooding- Influence of Contamination Source and Soil Properties**

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Antimony (Sb) is a toxic and potentially carcinogenic metalloid, which is listed as a pollutant of primary concern. Despite its increasing production over the last years, environmental concerns about Sb have emerged only recently and many aspects of its biogeochemistry remain unclear. When mobilized from contaminated soils to water, Sb poses a potential threat to surrounding environments and might become bioavailable. Soil Sb can be very mobile under flooded conditions, which are expected to occur more frequently in the future due to climate change.

Here, we incubated six Sb-contaminated soils under flooded conditions in mesocosms, which allowed for soil, porewater, surface water, and head-space sampling over an incubation time of three months. This talk focuses on the soil porewater, which was analyzed for Sb speciation, Sb size fractionation, and a series of explanatory parameters.

The study soils differ in their contamination source and properties including organic matter, pH, iron, manganese, and sulfur concentrations, which led to contrasting Sb release patterns in the soil porewater. In shooting range soils, highest porewater Sb concentrations were observed immediately after flooding through the release of easily soluble, dissolved anionic Sb(V). The Sb release pattern of mining-polluted soils differed greatly with contrasting soil properties: in inorganic Fe-rich soils, Sb release was mainly driven by co-release with reductive dissolution of iron-hydroxides, while the drivers behind Sb release in the organic soil are more complex.

Size fractionation of porewater Sb showed that the majority of Sb was present as very small, dissolved ions, meaning that colloidal transport only played a minor role in Sb mobility. Size exclusion chromatography analysis nonetheless revealed an association of Sb with Fe-oxides and organic matter, which increased with time after flooding. Interestingly, Sb-bearing particles >0.45µm were only measured in one organic mine-impacted wetland soil.

These results demonstrate the complex biogeochemistry of Sb, which underlines the difficulty of risk assessment of Sb-contaminated sites. By implementing new analytical methods and including soil, water, and air, this study enables a more comprehensive picture of Sb geochemistry in flooded soils and contributes to improved prediction of Sb behavior in the environment to better manage contamination events under changing climate regimens.

### **3.11.A.T-03 Understanding the multiple fates of rare earth elements (REEs) in abiotic and biotic compartments of the St. Lawrence River, Canada.**

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The demand for rare earth elements (REEs) is increasing due to their numerous applications, including low-carbon energy and digital technologies. Canada owns one of the largest REE deposits worldwide and has started exploiting its first mine in 2021. Combined with the intensification of urban development and agriculture, and climate change effect on browning and precipitations patterns, we can anticipate an increased mobilization of REEs to freshwater ecosystems that supports important ecological services and biodiversity. Therefore, there is a need to gain an integrated understanding of the fate of these metals in the abiotic (water, sediments) and biotic (invertebrates, fish) compartments of the fluvial landscape. Our recent work showed that REEs in the surface waters of the St. Lawrence River were increasingly associated with colloids and particles along the upstream-downstream gradient, which was linked to the terrestrial organic-rich inputs from its main tributary draining REE-rich geology, the Ottawa River, and which was further reflected in the relative distribution of light and heavy REEs. Here, our main objectives are to assess their accumulation and relative distribution in the biotic and abiotic compartments from the same river, and to identify the related environmental or biological predictors to understand their global fate at the ecosystem scale. Sediments were the most important sink for REEs and their accumulation was related to smaller grain-size and was relatively enriched in middle REEs, probably due to their association with Fe and Mn oxides. Uptake by both invertebrates and fish depended on species, feeding behavior and trophic position. In addition, organisms with more diversified diet, such as a combination of sedimental organic matter, plankton, and invertebrates, tended to accumulate more REEs than more pelagic or piscivorous organisms. In addition, our results showed that amphipods accumulated seemingly anthropogenic Gd around Montreal area and that, although walleye and sauger did not accumulate important concentration of REEs, their accumulation was directly correlated with the free ion form of REEs in surface water. These findings highlight major differences in REE concentration and composition among biotic and abiotic compartments of a large river, suggesting that sediments are the most susceptible to respond to future contamination and this this response is likely to decrease going up in the trophic chain.

### **3.11.A.T-04 Ecotoxicology of Rare Earths in aquatic systems: Integrated overview of fate and behavior of Rare Earth in river, their trophic transfer and environmental impacts in a Life Cycle Assessment perspective**

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Rare Earths are a group of 17 metals including the lanthanide series, Sc and Y. They are used in high-tech devices. Anthropogenic use is disrupting the biogeochemical cycle and enrichments of La, Ce, Nd, Sm and Gd are already detectable in waters. The current literature on REE-associated health effects is mostly confined to 3 REE, thus the information for several REE remains relatively scarce, notwithstanding their growing industrial utilization and, hence, environmental spread and human exposures. However, evidence gathers that REEs must be acknowledged as new, emerging contaminants with manifold ways of entry into the environment. The objectives of the project were : linking the speciation of REEs to their biological effects, combining field observations with laboratory experiments, to follow a tiered and interdisciplinary approach from field observations through screening to focused experimental manipulation under increasing environmental realistic conditions and developing an AOP framework and integrate this into a life cycle perspective.

ECOTREE represents a holistic and interdisciplinary proposal outside a mining context covering large parts of the REE life cycle, from the geochemistry of anthropogenic REEs, their dynamics in (waste)water systems, their speciation and related bioavailability, their trophic transfer and environmental impact assessment at different biological levels and include a Life Cycle Assessment perspective. The approach proposed in this project combines field observations and realism-checked laboratory experimentations to ensure that speciation, bioavailability, bioaccumulation and toxicity pathways for REEs were studied in realistic conditions on 5 living organisms representative of freshwater systems, allowing to better extrapolate of the laboratory results to field conditions.

The results obtained suggest erosion of soils as the main source of particulate REE in the two rivers, although a Nd anomaly of industrial origin occurred in the particulate and coarse colloidal fractions of the industrialized river basin. Toxicity of the series of REEs could not be correctly evaluated using the exposure nominal concentrations for algae and *Daphnia magna*, but on the concentrations of free ions that for some REEs changed during exposure time. The calculated EC<sub>50</sub> values ranged from tens of nanomoles for La, Ce, Pr, Sm, Eu, Gd, Tb and Dy to (sub)micromolar for the remaining elements with common biological modes of action.

### **3.11.A.T-05 Periphyton Is a Unique Ecological Niche Playing Critical yet Underrated Roles in Aquatic Mercury Cycling and Bioaccumulation**

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As a global pollutant, mercury (Hg) cycles between air, water, soil, and sediment involving transport and transformation of various Hg species, forming a complicated biogeochemical Hg cycle. Mercury poses adverse health effects to millions of people worldwide, primarily due to production of methylmercury (MeHg) in aquatic environments and bioaccumulation of MeHg along the food chain. Despite global effort to reduce Hg emission for alleviating its health risk, Hg time trends in fish often do not agree with concurrent decreasing trends in atmospheric Hg deposition to aquatic environments. This divergence suggests the current incomplete understanding of the biogeochemical processes controlling MeHg production and bioaccumulation in aquatic ecosystems. Although sediment is long known as the primarily site for Hg methylation, recent studies suggest the enhanced Hg methylation in periphyton, a unique algae-bacteria synergistic community and ubiquitous food web base in various aquatic environments and thus playing a critical role in MeHg bioaccumulation. Our effort here is to provide a summary of our previous work on the effects of periphyton on MeHg distribution and bioaccumulation and the literature on Hg methylation in periphyton, from the perspective of understanding the potential mechanisms underlying the enhanced Hg methylation and the implications of periphyton as a major source for MeHg bioaccumulation. Our work and others, albeit the number of studies being limited, have indicated that periphyton is conducive to Hg methylation with dissolved organic matter playing a critical role and periphyton-produced MeHg can have a major impact on aquatic MeHg cycling and bioaccumulation. Future microscale studies to elucidate the mechanisms of the coupled algal and bacterial Hg and C metabolism regulating Hg methylation in periphyton and macroscale investigations to comprehensively evaluate the role of periphyton in environmental distribution and bioaccumulation of MeHg are both urgently needed.

### **3.11.B Fate and Toxicity of Metals: Recent Scientific Advancements, Challenges for (Data-Poor) Metals, and Application to Environmental Regulations**

#### **3.11.B.T-01 Internalization of Organically Complexed Copper by a Coastal Dinoflagellate in Synthetic and Natural Seawater Samples**

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Copper (Cu) is by far the most studied metal concerning the effects of organic complexation on metal bioavailability, and it is widely accepted that the presence of organic matter has a protective effect on Cu uptake and toxicity, by decreasing the concentration of free metal ions. However, this idea is based in laboratory experiments either performed at higher Cu concentrations than those that are environmentally relevant, or in experiments that used synthetic chelates (such as EDTA) to control for Cu speciation. The present experimental work was designed to test if Cu bioavailability actually depends on the free ion concentration at an environmentally relevant concentration of Cu (20 nM), using both synthetic and natural samples in the absence of synthetic chelates. As synthetic samples, artificial seawater was amended with fulvic acids or Marine-DOM extracted by ultrafiltration. Natural samples were collected from a mining-impacted coastal lagoon (Mar Menor, SW Spain), presenting dissolved Cu concentrations ranging from 3.5 to 14 nM. The samples were enriched in <sup>65</sup>Cu (15 nM) in order to study the short term internalization of Cu by the coastal dinoflagellate *Prorocentrum micans*. Results show that net Cu internalization by *P. micans* is higher than that expected on the basis of Cu speciation (either analysed by anodic or cathodic stripping voltammetry). The results are consistent and occur in all samples, both the synthetic and the natural ones, suggesting that organically complexed Cu can be accessed by marine phytoplankton. This work challenges the applicability of current bioavailability models for Cu in coastal waters.

#### **3.11.B.T-02 Concentration Addition or Independent Action: Which Model Better Predicts Aquatic Toxicity of Environmentally Realistic Metal Mixtures?**

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Risk assessment of chemical mixtures is conveniently regulated following the concentration addition (CA) model. A more accurate alternative for chronic metal mixture toxicity is provided by the independent action (IA) model. A quantifier to assess the deviations of observed toxicity from toxicity predicted with CA at low effect levels is called the MIF (Mixture Interaction Factor), which indicates a trend toward synergistic (MIF<1) or antagonistic (MIF>1) interactions, relative to CA, if present.

This study aims to test the hypothesis that IA generally predicts better metal mixture toxicity than CA. In addition, it will, based on theoretical and mathematical considerations, test the hypothesis that the MIF increases with the number of metals in the mixture. This work is part of the comprehensive Eurometaux MEED (Metals Environmental Exposure Data) program, as project 5.

Ag, As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, and Zn were selected for testing as they were estimated to be the major contributors to mixture toxicity for *Raphidocelis subcapitata* in European freshwaters. For this species, it was estimated that in a mixture experiment, five metals are usually sufficient to explain 90% of the toxicity of the risk of the whole mixture. Therefore, 3 environmentally relevant groups of the above-mentioned 10 metals were selected for testing (i. As-Cu-Pb-Ni-Cd, ii. Mn-Zn-Ba-Cr-Cd, iii. As-Zn-Pb-Ag-Cu). Each experimental design consisted of testing simultaneously the 5 individual metals separately and in mixtures. The set of mixtures consisted of a binary (2 metals), a ternary (3), a quaternary (4) and 2 quinary (5) combinations. The experiments were conducted following an equitoxic ray design based on EC10 values, with one additional quinary mixture at environmentally relevant concentration ratios.

Overall, the two models showed rather similar results, with no clear trend toward synergistic or antagonistic interactions relative to the models. However, at environmental and regulatory more relevant concentrations (i.e., at 10% effect level), CA generally overestimated the mixture toxicity, as confirmed by the MIF values being all above 1, with an average MIF of  $1.59 \pm 0.59$  ( $\pm$ : SD). Finally, the MIF did not increase with the number of metals in the mixture. Possibly, the toxicity of the metal mixture (and the magnitude of the deviation from CA) is more influenced by the relative concentration of the metals in the mixture.

### **3.11.B.T-03 Investigating Population versus Individual Sensitivity of *Lymnaea stagnalis* to Nickel (Ni)**

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*Lymnaea stagnalis*, known as a particularly sensitive species to various metals, exhibits inconsistent responses across different studies regarding its sensitivity to nickel (Ni). Understanding the variability in the snail's sensitivity to Ni remains pivotal for effective risk assessment but poses a challenge due to the lack of a clear explanation for its toxicity mechanism. This study aims to create an experimental dataset which allows for the calibration of a DEB model to describe the toxicity of Ni to *L. stagnalis* and the validation of extrapolations to the population level using a DEB-IBM.

To examine the effect of Ni on different life stages of *L. stagnalis*, we conducted a series of 28 day toxicity tests. These experiments assessed the impact of Ni on endpoints such as growth, ingestion, and reproduction of the snails. Ultimately, these experiments revealed a stark contrast in sensitivity between juvenile and adult snails. While juveniles displayed sensitivity at relatively low Ni concentrations (11 µg/L), adult snails remained unaffected up to 166 µg/L. This divergence in individual level sensitivities prompted further population level experimentation.

During an extended 133-day exposure, populations of *L. stagnalis* showed significant decline in biomass and reproduction at 79 µg/L of Ni. These results call into question the protective efficacy of traditional reproduction tests for higher levels of biological organization. The discrepancy between individual and population responses highlights the need for a better understanding of the mode of action of Ni toxicity in *L. stagnalis*. The results of this population level test can be used as a potential validation for the existing *L. stagnalis* DEB-IBM, which incorporates population dynamics (e.g. competition for food) that do not impact standard individual level tests. Further refining population simulations may also allow for insights into the mode of action of Ni toxicity to the snails, which could not be determined based on individual testing.

Toxicity tests have demonstrated differing sensitivity between adult and juvenile *L. stagnalis*. Extended population exposure showed significant effects on population biomass and reproduction at lower concentrations than observed in adult reproduction studies. Further work is needed to more fully understand the mechanism of Ni toxicity to *L. stagnalis* and to investigate what factors are most impactful the relationship between individual and population sensitivity.

### **3.11.B.T-04 Estimating and Using Ambient Background Concentrations in Surface Waters for Nickel in Europe**

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Naturally occurring substances such as nickel will always be present at some level in the environment, although the concentrations at which they are present may be increased by anthropogenic activity. Recent activities on substance prioritisation and Environmental Quality Standard (EQS) derivation under the European Water Framework Directive (WFD, Commission Directive 2000/60/EC) have demonstrated that the use of assessment factors on HC5s in deriving metals EQS can result in values that are within the range of ambient background concentrations (ABCs) and lead to potential non-compliance even in some relatively pristine areas. EQS derived in this way may provide little or no ecological benefit, but may drain limited regulatory resources. Taking account of local background concentrations due to the naturally occurring nature of nickel may therefore be appropriate in some situations and inline with current guidance.

We have developed an approach for identifying situations where local background concentrations should be considered, and for deriving them if required, that is straightforward, transparent, scientifically defensible, and readily implementable by non-experts.

A checklist was developed to identify whether there is a potential need to derive a local background concentration at a site and an assessment was performed from a collated regulatory freshwater monitoring dataset of 545,577 samples, covering 21,092 sites in 26 countries. Surface water data are the most appropriate source to use for the derivation of background concentrations due to their direct relevance and likely availability to the assessors needing to derive local background concentrations. Data from sites that are relatively close to those for which background concentrations are required and are not significantly impacted by anthropogenic disturbances is recommended to use as the source of background concentrations.

Following the completion of the tiered risk assessment, three countries (Cyprus, France and Spain) were identified to support case-studies and the methodology for Tier 3 failures was applied. For both Cyprus and France a number of Tier 3 failures could be resolved by applying this methodology, and therefore country-wide compliance rose. This presentation will include example sites from these case studies showing the application of the derived methodology.

### **3.11.P Fate and Toxicity of Metals: Recent Scientific Advancements, Challenges for (Data-Poor) Metals, and Application to Environmental Regulations**

#### **3.11.P-Mo133 Tracing Potential Emissions from Corrosion Protection Systems for Offshore Wind Farms in the Marine Environment**

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In order to combat climate change, the share of renewable energy production must increase drastically. Offshore solutions are gaining more and more importance and the number of offshore wind farms is growing steadily. As of 2021, 28 GW of offshore wind energy was installed in Europe and 55.9 GW worldwide. The UN has set an even higher / more ambitious goal, to increase the global offshore wind capacity to 380 GW by 2030.

The impact of these offshore constructions on the marine environment remains unclear in many aspects. In fact, little is known about potential chemical emissions from corrosion protection systems such as galvanic anodes composed of AlZnIn alloys, used to protect offshore structures. Those anodes are designed to corrode in place of the structural steel, resulting in the continuous emission of metals (e.g. >2000 kg Al-anode material per pile for the lifetime of 25 years) into the marine environment. In a previous study the elements Al, Zn, Cd, Pb, Ga and In were identified as potential tracers for offshore-wind-induced emissions.

Seawater, sediment, and blue mussels were sampled from offshore wind farms in the German Bight and analyzed via inductively coupled plasma mass spectrometry to obtain multielement and isotope data. In the seawater samples an accumulation of metals along the residual current was observed in some years, and for sediment samples some local hotspots were identified. However, Sr isotope data suggests a large influence of sediments from the tributaries. Blue mussels did not show elevated levels of Cu, Zn and Pb compared to the North Sea and to international health standards, however, no accumulation of In and Ga were observed. In conclusion, multielement datasets can be used to trace emissions from offshore wind farms. In particular, the TCEs Ga and In are promising tracers, and first hints for emissions from corrosion protection systems were found in seawater and sediment samples. However a clear source assignment is currently difficult, because source differentiation remains challenging and demands a continuous monitoring of old and new tracers

#### **3.11.P-Mo134 Influence of the initial speciation of platinum and palladium on their bioavailability to a green alga**

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The unique properties of platinum-group elements (PGE) have led to many applications for these metals. Consequently, multiple emission pathways have been investigated and a relation was firmly established between global demand for these metals and their subsequent release in the environment. It is therefore necessary to better understand the potential risks associated with PGE. In classical metal toxicity studies, it is often assumed that thermodynamic equilibrium in the exposure medium is reached rapidly. This however, may not be true for platinum (Pt) and palladium (Pd) due to their inherently slow kinetics. Indeed, the water exchange rate - a key indicator of reaction kinetics - for most transition metals is in the order of  $10^{-7}$  to  $10^{-10} \text{ s}^{-1}$ , while for Pt and Pd, the rate constants are orders of magnitude higher ( $\sim 10^3 \text{ s}^{-1}$ ). If the equilibrium period is long relative to the total exposure time, aquatic organisms may be mostly exposed to the complexes present at the start of the test rather than those expected to be present at equilibrium (or to a mixture of the two).

In this project, we examined the accumulation of five Pt and Pd complexes ( $\text{Pt(IV)Cl}_6^{2-}$ ,  $\text{Pt(II)Cl}_4^{2-}$ ,  $\text{Pt(II)(NH}_3)_4^{2+}$ ,  $\text{Pd(II)Cl}_4^{2-}$  and  $\text{Pd(II)(NH}_3)_4^{2+}$ ) by the unicellular green alga *Pseudokirchneriella subcapitata* and their subsequent toxicity after 96 h of exposure in USEPA medium. An investigation of the stability of the tested complexes was carried out by spectrophotometry and reaction kinetics calculations. Our results point at the rapid dissociation of Pd complexes leading to the formation of  $\text{Pd(OH)}_2$  in the case of  $\text{Pd(II)Cl}_4^{2-}$  and the formation of a mixture of  $\text{Pd(NH}_3)_2^{2+}$ ,  $\text{Pd(OH)}_2$  and  $\text{Pd(NH}_3)_4^{2+}$  in the case of  $\text{Pd(II)(NH}_3)_4^{2+}$ . Similar Pd accumulation was measured for both studied complexes but  $\text{Pd(NH}_3)_4^{2+}$  was slightly more toxic, maybe due to the presence of  $\text{Pd(NH}_3)_2^{2+}$  and  $\text{Pd(NH}_3)_4^{2+}$ . On the other hand, all three Pt complexes tested resulted in similar accumulation by the algae but only  $\text{Pt(IV)Cl}_6^{2-}$  led to a significant growth inhibition. Interestingly,  $\text{Pt(II)(NH}_3)_4^{2+}$  was shown to be stable in test medium for at least 96 h whereas  $\text{Pt(II)Cl}_4^{2-}$  was much less stable with indications of dissociation occurring within less than 24 h. This suggests that, initial speciation of Pt(II) complexes does not affect toxicity over the concentration range tested (0 - 150  $\mu\text{g/L}$ ). Furthermore, the surprising  $\text{Pt(IV)Cl}_6^{2-}$  toxicity was potentially linked to free radical species formed during the reduction to Pt(II).

### 3.11.P-Mo135 Knowledge gaps in thermodynamic data needed to predict the aqueous speciation of platinum-group elements

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The current tenet of metal bioavailability stipulates that the uptake and toxicity of a metal are not related to its total concentration in solution but rather to its free-ion activity (the other main toxicity modifying factors being hardness and pH). Ideally, the free-metal ion activity (or concentration) would be measured analytically. However, currently available analytical techniques do not allow for routine measurements as most are metal-specific, labour-intensive, subject to matrix interferences or lack sensitivity. On the other hand, aqueous metal speciation can be effectively predicted using the appropriate thermodynamic data (formation constants). Typically, metals will form inorganic complexes with ligands such as hydroxide, carbonate, chloride and fluoride ions. For many transition elements (e.g. Cu, Pb, Zn), thermodynamic data for these complexes are readily available and speciation can be calculated with a high degree of confidence. Even in the presence of polyfunctional heterogeneous natural organic matter (humic and fulvic acids), complexation can normally be estimated with reasonable confidence.

The growing demand for less traditional elements (e.g. technology critical elements) is driving environmental regulators to develop new criteria for environmental protection. However, these cannot be derived without the appropriate ecotoxicological data, and the production of such data requires basic knowledge of metal aqueous chemistry. We reviewed the available formation constants for platinum group elements (Ru, Rh, Pd, Os, Ir and Pt) and identified key missing data. Among these metals, palladium is the one for which the most comprehensive data set is available. At the other end of the spectrum, there are currently no thermodynamic data available for iridium. We also explored the use of Linear Free-Energy Relationships (LFER) to fill data gaps. These involve the establishment of empirical relationships between complexes. For example, the binding constants of the first hydroxo-complex of metals (MOH) are usually available and can be correlated with the formation constant with a more highly substituted complex (e.g.  $\text{M(OH)}_2$ ) or with another ligand (e.g.  $\text{MCO}_3$ ). LFER can thus be used to estimate missing data or to select a value when large discrepancies are observed among available values. Examples will be provided with a focus on Pt(II). Future research priorities will be discussed.

### 3.11.P-Mo136 Comprehensive Analysis of (Ultra)trace Elements in Seawater: Method Development, Dilution Studies, and Matrix Effects Investigation

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There is a growing need for the development of rapid and accurate methods to quantify trace and ultra-trace elements in complex matrices like seawater. The determination of such elements is often hindered by the lack of sensitivity, contamination, artifacts, spectral overlap, or memory effects. These challenges can be partially addressed by employing high resolution inductively coupled sector field mass spectrometry (HR-ICP-MS) in a clean room. In this study, using HR-ICP-MS, we assess various analytical approaches for directly measuring trace and ultra-trace elements in seawater. In particular, dilution factors of 10 to 3000 were tested to verify the consistency of the results throughout the concentration range. Additionally, we analyze the concordance of these results against pre-concentrating (ultra)trace elements and removing the sodium matrix with the seaFAST cation exchange method followed by HR-ICP-MS analyses. Matrix effects are investigated by using seaBlank samples and standard addition methods. The validation of the method was carried out using certified seawater material. The method developed within this work allows researchers to fast and accurately quantify (ultra)trace elements, including technological critical elements in complex matrix samples, such as seawater, marine sediments, and corals. This approach provides a valuable tool for expanding our knowledge of the fate of these elements in marine ecosystems and their potential effects on organisms.

### 3.11.P-Mo137 Metals and Particulate Matter in the Discharges Resulting from In-water Hull Cleaning Conducted via Remotely Operated Vehicle (ROV): Levels and Rates of Release into the Marine Ecosystem



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The increased utilization of remotely operated vehicles (ROVs) for in-water hull cleaning (IWC) has raised concerns regarding the environmental impact of IWC effluent discharge. This study aims to explore the characteristics of wastewater produced during IWC, specifically focusing on concentrations of suspended solids (SS) and metals, as well as their release rates and total environmental load. Notably, IWC effluents contain significant amounts of SS and metals, with copper (Cu) and zinc (Zn) being the predominant metals. These metals are mainly associated with fine antifouling paint particles, posing a potential risk of secondary pollution upon entering the marine environment. Although treatment systems have shown effectiveness in reducing SS and particulate metals, achieving complete removal of dissolved and particulate metals below ambient levels has proven challenging. To address this, the study suggests the implementation of multistage filtration systems based on particle size analysis, with an optimal filtration pore size for efficient effluent treatment. In summary, this research underscores the potential environmental risks associated with IWC activities. Given the strong affinity of metals towards particles in wastewater, the effective removal of particles emerges as a crucial factor in mitigating environmental stress at IWC sites. The release rate is an essential factor to predict the environmental concentration of contaminants and to assess the environmental risk posed by IWC. This manuscript will provide scientific foundation for the regulation and management of IWC to protect the marine environment.

### **3.11.P-Mo138 Speciation of Lithium, Manganese, Cobalt and Nickel in Fresh Water by Field-Ion Exchange Technique**

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Metal speciation is a key parameter to take into account when evaluating metal bioavailability in natural waters. The free ion activity (concentration) is especially of interest as being identified as the best proxy to predict metal accumulation and toxicity towards aquatic organisms. One way to achieve the determination of the free ion concentration is the use of thermodynamic calculation but it might become inaccurate in a complex and fluctuating environment, such as in a river. Another approach is the direct determination of the free ion concentration using analytical techniques, such as Ion Exchange Technique (IET). This approach consists of equilibrating a cation exchange-resin with the solution to be analyzed. The free metal ion concentration in solution is then proportional to the metal bound to the resin. If generally performed into the laboratory, the IET use can be extended into the field with the resin placed into a dialysis cassette. In the present study, we evaluated the use of the field-IET to determine the free ion concentrations of Co, Ni, Mn and Li present in freshwaters.

To that end, field-IET cassettes were prepared by placing ~30 mg of DOWEX 50W-X8 resin into dialysis cassettes made of low-binding regenerated-cellulose membrane of 20 kDa cutoff. This resin was firstly preconditioned using Ca and Mg concentrations of the Gave de Pau, which was the river of interest. The field-IET cassettes were then deployed in mesocosms (TotalEnergies facility, France) filled with the Gave de Pau river to be exposed to increasing Li (2, 10 and 20 mg/L), Mn, Co, and Ni (6, 30 and 60 mg/L) for 7 days.

Three days of deployment were required to achieve an equilibrium between the resin and the ambient water. Extended period of deployment led to a decrease in the metal bound to the resin, most probably due to the fouling of the dialysis membrane. The concentrations of Ca and Mg in ambient water were also found to influence the distribution coefficients of all studied metals, requiring the measurements of Ca and Mg in all analysed samples. Further analyses will be performed to compare the free ion concentrations using the field-IET and thermodynamic determination.

### **3.11.P-Mo139 Long-term feasibility assessment of in-situ heavy metal immobilization using calcium polysulfide in groundwater**

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The in-situ immobilization of high-concentration heavy metals (Cd<sup>2+</sup>, Zn<sup>2+</sup>) in groundwater using polysulfide (CaS<sub>x</sub>, CPS) was demonstrated for the field application. When CPS introduced into the aquifer, CPS solution descended rapidly and reacted with heavy metals in groundwater since the density ( $d = 1.27 \text{ g/cm}^3$ ) of CPS solution was greater than that of water. Both Cd<sup>2+</sup> and Zn<sup>2+</sup> in filed groundwater were rapidly reacted with injected CPS solution and effectively and instantly immobilized. The majority of heavy metals in complexly-contaminated groundwater generated metal sulfide precipitation in the field. The XRF, FE-EPMA and SEM/EDS results showed metal sulfide precipitates were clearly observed through the reaction between the CPS solution and heavy metals. In addition, microbial communities in groundwater were monitored using next-generation sequencing during the demonstration period. After CPS injection, the microbial diversity of groundwater was reduced, and sulfur-related bacteria were dominant in the groundwater monitoring wells which most of heavy metal were immobilized. This showed that microbial communities in groundwater also play important roles in of heavy-metal immobilization by CPS usage. Finally, an injection protocol optimized for various factors such as injection concentration, injection amount, and injection method of CPS solution for site-specific subsurface systems should be established to prevent clogging, re-mobilization and relocation of heavy metals.

### 3.11.P-Mo140 Occurrence, fate, transfer of lithium and its isotopes in freshwater ecosystems: implication for its environmental risk assessment

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The development of renewable energies and digital technologies requires critical raw materials including lithium (Li). The increasing demand (+3535 % by 2050 in EU) and uses of Li will lead to its release into the environment including freshwater ecosystems, which could potentially affect them. However, Li bioaccumulation and its potential biological effects towards aquatic species are currently unknown. Lithium can thus be considered as data-poor metal.

The present postdoctoral project aims to 1) determine Li exposure in freshwater ecosystems by analysing its environmental concentrations in abiotic and biotic compartments; 2) study the natural variations and fractionation of Li isotopes in a freshwater food web and assess the potential Li trophic transfer; 3) investigate the distribution of Li and its isotopes at tissue and subcellular levels and their potential hazard/ toxicity by analysing Li toxicokinetic in a freshwater bivalve through experimental and modeling approaches.

To tackle these issues, we have selected three study sites in France (Lorraine and Auvergne regions) having different geogenic (sandstone, marlstone, granite) and anthropogenic sources of Li (mining activity, battery recycling). The concentrations of Li that we have measured in the sediment and water were higher than French median concentration ( $3 \mu\text{g L}^{-1}$ ) by around 1,000-fold in the stream located in the former coal mining area, which represents the highest hotspot of Li currently known in France. The results also pointed out relatively high concentrations of Li in aquatic organisms. The aquatic plant *Myriophyllum spicatum* accumulates more Li ( $6.9\text{-}16.0 \mu\text{g g}^{-1}$ ) than the crustacean *Gammarus sp.* ( $1.5\text{-}7.4 \mu\text{g g}^{-1}$ ) and the bivalve *Corbicula fluminea* ( $2.4\text{-}3.0 \mu\text{g g}^{-1}$ ).

Isotope analyses are ongoing and will allow deciphering different sources of Li in aquatic systems. <sup>6</sup>Li isotope is supposed to be more biologically reactive than <sup>7</sup>Li because of its different mass. Thus, the investigation of <sup>6</sup>Li/<sup>7</sup>Li ratios will help to better understand Li bioaccumulation and subsequent biological effects. Produced data will be considered for Li environmental risk assessment.

### 3.11.P-Mo141 Adsorptive removal of Heavy metal in aqueous phase by biochar combined with biosulfur material

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Biochar has been used to remove heavy metals in aqueous phase as an adsorbent. This study was conducted to evaluate heavy metal removal efficiency in aqueous phase utilizing biochar (BC) combined with biosulfur (BS), the sulfur rich materials produced from landfill leachate treatment process. In order to manufacture combined biochar and biosulfur (BCBS), BC, BS, and distilled water (1:4:5, v/v/v) were mixed at the 25°C for 1 hour. Different mixing ratio (0.05, 0.1, 0.5%) of BCBS was mixed with solution containing 1,000 mg/L of Cd and Pb. The adsorption efficiency of Cd and Pb was ranged from 78% to 98% and the highest adsorption was observed when 0.1% of BCBS was applied in the heavy metal solution. The reaction rate and isothermal adsorption showed that the pseudo second order and Langmuir model were the best fit for describing adsorption of the both Cd and Pb. X-ray infrared techniques such as SEM-EDS, FT-IR, and XPS were also examined to verify the removal mechanism of soluble heavy metals. According to result of various X-ray infrared techniques, ionic format of Cd ( $\text{Cd}^{2+}$ ) and Pb ( $\text{Pb}^{2+}$ ) makes complexation with sulfide or sulfate at the surface of BCBS. Thus, ionic complexation or co-precipitation of ionic form of heavy metals and sulfur compounds were the main removal mechanism of heavy metals when BCBS was applied in the aqueous phase.

### 3.11.P-Mo142 A mesocosm study to assess selenium dynamics in a Mediterranean floodplain wetland

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The Tablas de Daimiel National Park (TDNP) is a floodplain wetland formed by Cigüela and Guadiana rivers in central Spain whose water levels have drastically dropped over recent decades because of overexploitation of freshwater resources, worsened by climate change, and that receives pollutant inputs from WWTP discharges and the runoff from surrounding agricultural lands. High selenium levels have been found in sediments and biota, which could be linked to the Se-rich soils in the area but also to anthropogenic pollution. Se speciation in the environment depends, among others, on pH and redox potential, and determines bioavailability of the element. The change of the hydrological regime that affects TDNP, with longer droughts and shorter flooding periods, could be influencing the Se speciation and its bioavailability. To study the processes of transformation and bioaccumulation of Se in the food chain, we have designed mesocosm trials simulating three hydrological regimes: (i) constant flooding with a water renewal mimicking the optimum TDNP hydrological regime ( $10 \text{ hm}^3/\text{month}$  for a flooded surface area of 1600 ha); (ii) drought-flood cycles, with complete refilling every three months, simulating the action of an existing dam at the wetland mouth, and (iii) prolonged drought, with complete water evaporation and full system reflooding. Mesocosms were set in 1000 L-containers with a 20-cm deep layer of sediment from the Park area with higher Se levels ( $4 \mu\text{g/g}$ ), 800 L water and three plant species (*Scirpus maritimus*, *Chara sp.* and *Typha domingensis*). Samples of each matrix have been taken every two months to analyze total Se through ICP-MS and Se species using HPLC coupled to ICP-MS. We

hypothesized that drought-flood cycles favor the mobilization of Se from sediments, so we expect bioavailability, and therefore bioaccumulation in plants, to be highest in the last scenario immediately after reflooding events. Study funded by the Spanish Organism of National Parks (ref. SPIP2021-02790).

### 3.11.P-Mo143 Heavy Metals Extraction From Industrial Sludges

*Sam Li, NUS, Singapore*

Globally, large volumes of industrial sludge are produced throughout the municipal wastewater treatment process. Proper disposal of industrial sludge is required to avoid secondary pollution. A range of metals are usually present in the sludge, some of which are harmful to human health and others may have a high recycling value. As a result, eliminating the metals from sludge ash will lessen its toxicity and produce some useful products. This study concentrated on a raw material known as CuPbZn (CPZ) sludge. By using the well-known BCR sequential extraction procedure, 87% Cu and 37% were determined as the mobility fractions of these metals. Subsequently, three extraction techniques based on the use of HCl and EDTA as extraction reagents followed by electro dialysis was evaluated, and the deposit on the electrode was collected and examined. According to experimental results, the highest extraction rates of Cu and Ni were obtained by using 1M HCl as extraction medium (75.69% and 86.00%, respectively). Cu and Ni were only partially extracted using either 6% EDTA or 2M HCl + 6% EDTA as extraction solvents. Within eight hours of electro dialysis, Cu could be nearly completely extracted (>99%) from the sludge for all three types of extraction solutions. By extending the electrolysis period and forming agglomerates through coagulation, free nickel in 1M HCl extraction solution could be extracted (84.70% recovery rate). The results obtained demonstrated that appropriate combinations of sequential extraction with electro dialysis could provide more accurate analytical data for evaluating fate and toxicity of metals.

### 3.11.P-Mo144 Accessing the Past: How a Sediment Core can Help to Reveal Anthropogenic Impacts of Technology-Critical Elements.

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Technology-critical elements (TCEs) have undergone a dramatic increase in industrial applications in recent years. Still, many studies and legal directives only consider legacy pollutants like Cd, Pb and Zn as elements of interest. With the expansion of high-tech applications, the environment is becoming exposed to TCEs. Even though these potential emerging contaminants are getting more attention recently, the applications causing the release of TCEs into the environment already exist, making it difficult to assess current anthropogenic inputs or to access background values. A promising approach are sediment cores, which can be resolved, if deep enough, into recent and anthropologically un-impacted background levels.

A sediment core was taken in August 2022 in the Baltic Sea. The core was cut into 79 slices (1 cm thick), each of which was freeze-dried and milled. Every second slice was subsequently introduced to microwave-assisted acid digestion and analyzed for the mass fractions of 47 elements using inductively coupled plasma tandem mass spectrometry (ICP-MS/MS). The other core slices were used for age determination by <sup>137</sup>Cs and <sup>210</sup>Pb dating.

Age determination showed that slices from 30 cm and deeper are about 60-70 years and older, which allows the calculation of background values for the Baltic Sea from around 1950-1960. For legacy pollutants like As, Cd and Pb average mass fractions of 9400 µg kg<sup>-1</sup> ± 900 µg kg<sup>-1</sup>, 970 µg kg<sup>-1</sup> ± 80 µg kg<sup>-1</sup> and 23000 µg kg<sup>-1</sup> ± 6000 µg kg<sup>-1</sup> were found within 30 cm to 79 cm of the sediment core, respectively. Ga, as an example of a new emerging contaminant, due to its use in corrosion protection systems (galvanic anodes) of offshore wind farms was found with average mass fractions of 12600 µg kg<sup>-1</sup> ± 400 µg kg<sup>-1</sup> within the same lower part of the sediment core.

This study aims to provide background mass fractions for the Baltic Sea environment for new emerging contaminants, which are currently missing for a sound assessment of the role of potential emission sources into the marine environment such as offshore renewable energy production.

### 3.11.P-Mo145 Impact of the Heavy Metal Chemical Speciation Method selection and its Implications on the Results of the Health Risk Assessment

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Heavy metals due to their persistence and long-term adverse effects on the environment and human health became a major global issue. Risk assessment evolved into an important tool in evaluating the probability of the adverse effects occurrence for the environmental and human health due to the anthropogenic pollution and in numerous countries is nowadays a mandatory step in environmental decision-making.

As the heavy metal (HM) content is the major constituent of the risk assessment calculations, the choice of method that determines their chemical speciation and related concentrations in defined environmental samples together with HM fate and

metabolism, becomes a paramount issue. Relying solely on the total HM contents can overestimate the real risk values, while the bioavailable fraction of HM is considered to be more reliable in assessing risk of the adverse health effects occurrence.

In this study the differences in the HM contents obtained using various extraction methods were investigated to provide the input values of HM contents in following health risk assessment calculations. Following chemical speciation methods, commonly used in the risk assessment research, were used in our study to obtain HM contents: total content (TC), 3-stage Sequential Extraction (BCR), Simple Bioavailability Test (SBET), Standardized Bioavailability Test (BARGE), ISO Bioavailability Test (17924:2018), USEPA Bioavailability Test (Method 1340), and Physiologically Based Extraction Test (PBET). Concentrations of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Sn, Tl, and Zn were measured using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) in a diverse range of environmental samples, including soil, vegetables, fruits, cereals, and teas, both natural and Certified Reference Materials (CRMs).

Based on the results of HM contents from various chemical speciation methods the risk values for investigated groups of environmental samples will be calculated using Monte Carlo simulations. The analysis will encompass an examination of how choice of speciation method may affect the obtained HM contents and further how these values influence the risk values and following regulatory environmental decisions.

The results of the study will provide the new insight into the unification of risk assessment methodologies to support performing environmental regulations relevant to exposure to metals.

### **3.11.P-Mo146 Assessing the effects of iron smelters on soil quality utilizing earthworms**

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Pollution of the natural environment is not a new concept, but it is still a pressing matter worldwide. This study aimed to assess which metals are introduced into the surrounding soil environment (at different distances from the source point of pollution) by iron-smelting plants and the effect thereof on earthworms as bioindicator species.

The metal analysis performed on the collected soil samples indicated elevated levels of metals close to the smelting facilities and the concentrations decreasing as the distance to the iron smelting facility increased. The concentrations of Fe and Al were the highest of all metals tested in all three sampling locations.

The earthworm bioassays and avoidance behaviour test results paint a similar picture. The growth data confirms that at one of the sites, earthworms showed a positive growth rate over the experimental period compared to the other two sites investigated.

Results regarding reproduction indicated that the concentration of metals did not affect the adult earthworms and their ability to reproduce, but it did influence the survival rate of juveniles after hatching. Avoidance-behaviour results also correlate with the previous results, with the earthworms showing some avoidance, but not enough to be considered actively avoiding the soil and statistically significant.

It was concluded that some of the metal concentrations seen are elevated, but in most cases, they are not in high enough concentrations to be considered polluted, or if they are characterised as pollutants, the bioavailability of the metals might be a limiting factor.

### **3.11.P-Mo147 Metals Concentrations and Bioaccessibility in Soils at Parks and Playgrounds in Fort McMurray, a Major Oil and Gas Hub in Canada**

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Characterization and mapping of metals in soils is an important first step in the evaluation of ecological and human health risks towards the overall achievement of the United Nation's Sustainable Development Goal 3: Good Health and Well-being. Results from investigations conducted in parks and playgrounds in Western Canadian cities including Vancouver, Victoria, Edmonton, and Calgary indicate some metals (e.g., As, Cu, Pb, Zn) in soils exceed the Canadian Council of Ministers of the Environment (CCME) soil quality guidelines at few locations. Metal concentrations reflect the regional natural-occurring differences in concentrations and physicochemical properties or anthropogenic sources such as proximity to industrial activities, pesticide treated wooden structures, and heavy traffic corridors. Fort McMurray, an urban service area in the Regional Municipality of Wood Buffalo is perched above the Athabasca bitumen sands formation. This region is one of Canada's major hubs of oil and gas production. Sixty soil samples collected from 20 parks and playgrounds in Fort McMurray were analyzed for total metals by x-ray fluorescence. The mean metal concentrations (in mg/kg dry weight) were As: 1.0, Cd: 2.8, Cr: 31.8, Cu: 9.7, Pb: 8.0, Ni: 10.2 and Zn: 55.6 mg/kg which were all below the Canadian Council of Ministers of the Environment (CCME) soil quality guidelines for park land use. Ongoing work include determining soil physicochemical properties such as pH and total organic carbon content and in vitro bioaccessibility. The results will be evaluated to ascertain how industrial activities including oil and gas production in the community impact metal distribution in the parks studied. The

Fort McMurray data will be compared to results from the other Canadian cities listed above. The relationship between soil physicochemical properties and metal bioaccessibility and how they impact the risk associated with incidental soil ingestion during playtime at the parks will be explored. The estimated daily intake will be determined for each element using CCME human health risk assessment protocols incorporating the bioaccessibility data and the overall hazard index will be calculated. Finally, recommendations on limiting exposure to potentially contaminated soils during playtime at the parks will be developed with the aim of achieving the United Nation's Sustainable Development Goals.

### **3.11.P-Mo148 Leaching of Rare Earth Elements in Soil Samples from Electronic Waste: La, Nd and Dy as case study**

**Vernon Somerset, Martin Makombe and Charlton Van der Horst, Cape Peninsula University of Technology, South Africa**  
The dumping of waste electrical and electronic equipment (WEEE) and its recycling commonly known as "urban mining" has caused undesirable impacts on the environment. Many of these products are rich with rare earth and hazardous elements that end up in rubbish dumps and recycling centres. Harmful metals are exposed and leached into the environment. The fate of rare earth elements (REEs) through the disposal of electronic waste and urban mining into the environment has caused pollution and pose a threat to human health and the aquatic ecosystem. In this work, we report the results of several microcosm studies using conditioned soil to investigate the leaching of REEs in lab-scale experiments. The effective leaching of specific rare earth elements (La, Nd and Dy) were conducted under different experimental conditions. The effects of major variables on REE leaching were evaluated, which included lixiviant type and concentration, time, stirring speed, pH and solid to liquid ratio. The temperature was kept constant during all experiments at  $23 \pm 2$  °C. The leaching efficiency of La, Nd and Dy was found to be significantly dependent on acid concentration and leaching time. The best leaching efficiency was obtained with 1 M HCl with a leaching time of 30 minutes, 300 rpm stirring, 50 g/L solid to liquid ratio. It was found that the leaching efficiency of 69%, 75% and 77% was achieved for La, Dy and Nd, respectively. In speciation studies, the results showed that 90% of REEs were obtained from acid-soluble fraction and residual fraction. The study is currently investigating the availability of the fractions, chemical behaviour in soil under environmental conditions, and the uptake of the REEs by biota.

### **3.11.P-Mo149 Assessment of Metal Content in Urban Synthetic Soils Made of Crumb Rubber Including Playgrounds and Synthetic Football Fields**

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The disposal of end-of-life tires (ELTs) is an important problem in solid waste management. In the last decades, the main recycling route is the transformation in crumb rubber, which is largely used in playground and sports fields as infill. This microplastic material presents high metal concentration including toxic and heavy metals. Crumb rubber infill of artificial turf fields represents the largest individual source of intentional microplastics in the environment. The new EU regulation 2023/2055 bans granular infill for use on synthetic sports surfaces but these kinds of facilities will still survive for at least a transitional period (8 years).

In this study, element and metal distribution in a high number of real samples taken from different urban places, including playgrounds and sports fields from Santiago de Compostela (NW, Spain) and surroundings, is presented. Most of the samples were collected outdoors but some indoor samples were included (e.g. airport playground). Some synthetic and natural surface alternatives (cork, sand, soil, and thermoplastic pellets) were also collected and analysed. 28 elements including metals (Ag, Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, In, K, Li, Mg, Mn, Mo, Na, Nb, Ni, Pb, Se, Sr, Ti, Tl, U, V, and Zn) and 4 metalloids (As, B, Sb, Si) were considered. The samples were subjected to microwave digestion followed by ICP-MS analysis. The results shown high concentrations. Zn levels were extremely high, with values between 1-2 % in all the crumb rubber samples. Heavy and toxic metals such as Pb and Cr were found in most samples reaching values above 20 ppm. Some high values were found in root retainers made from crumb rubber, which could facilitate the plant intake of the toxic metals. The data were subjected to ANOVA analysis showing in some cases statistical differences between metal content in playgrounds and football fields. Other materials showed to be a safer alternative from a chemical point of view regarding toxic metal content.

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### **3.11.P-Mo150 Characterization of Nanoparticulate Metals Generated from Transportation, Tire Wear, and Urban Wildfires**

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Vehicular transportation generates nanoscale tire wear particles (TWP) and brake dust, collectively referred to as road dust sediments (RDS). RDS can contain high concentrations of Nanoparticle (NPs)-associated metals (Cd, Cu, Co, Cr, Ni, Pb, Ni, and Zn), as well as microplastics. NPs are highly reactive due to their high surface area to mass ratio, which can facilitate the environmental and biological uptake of trace metals, making their quantification in environmental systems essential. TWPs are

sources of Zn and Cr to the environment, although it is difficult to distinguish TWP-associated metals from other anthropogenic or geogenic sources. Characterizing RDS for metals associated with TWP in environmental samples presents many analytical challenges, due to the ubiquity of microplastics in the environment, associated with particle sizing instrumentation, and analytical interferences due to the complexity of environmental samples.

We capitalize on the high sensitivity and elemental specificity of single particle Inductively Coupled Plasma-Mass Spectrometry (spICP-MS) to identify and quantify metal containing NPs in RDS runoff from precipitation events. Using crumb rubber particles and established Zn and Cr content of tires, we determined particle size distributions of TWPs. We applied this methodology to precipitation runoff samples and crumb rubber that had burned during a wildfire, with the latter showing an alteration in the Cr particle size distribution. Results suggest a difference in the form of Cr in TWP post-combustion. We measured the RDS samples for 28 total metals, including metals associated with catalytic converters and vehicle brake pads such as Pt and Rh. We also analyzed samples for 8 NP associated metals. Particle size distributions were used to distinguish between dissolved and particulate metals, which can have different modes of toxicity. We found that RDS samples contained high concentrations of NP-associated Cu, Pb, Cd, and Zn. This study represents a novel application of spICP-MS for RDS characterization in complex environmental samples. The result will lead to more accurate quantification of metal containing NPs in RDS and other matrices, which is essential for understanding environmental toxicity potential from contaminants including microplastics and toxic metals.

### **3.11.P-Mo151 Analysis of Drinking Water to Soil With a Single Method on ICP-MS and an Auto-Dilution System**

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ICP-MS, which can measure multiple elements simultaneously with high sensitivity, is used in many environmental laboratories to quantify hazardous elements in environmental samples. As environmental laboratories often need to analyze many samples on a daily basis, it is very important to increase the efficiency of work and reduce the demands on lab staff through automation. To improve workflow efficiency, a new application for environmental analysis of drinking waters through to soils is described. Utilizing new capabilities of inline auto-dilution for ICP-MS and ICP-OES, consisting of three main functions.

The first is the ability to make calibration curves from a single stock solution, which significantly reduces the preparation time for standard solutions. The second is the ability to automatically dilute samples directly prior to measurement, reducing the preparation time for samples with high matrix concentrations such as sediments and soils. The third is the ability to automatically dilute and re-measure samples immediately when the concentration of target elements exceed the calibration range or the internal standard elements exhibits suppression or enhancement.

This makes it possible to prevent post-analysis rework by avoiding manual dilution of affected samples. Inline auto-dilution not only reduces manual labor, but also avoids the risk of human error and contamination, further improving laboratory productivity. This poster will also report on the evaluation of dilution accuracy, precision and long-term stability using certified reference materials.

### **3.11.P-Mo152 Comparison of Voltammetric Techniques to Determine the Chemical Speciation of Dissolved Copper in Mar Menor Lagoon**

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Las concentraciones totales de metales no son predictores adecuados de su biodisponibilidad, por lo que se debe considerar la especiación química de los metales. La especiación de Cu disuelto en agua de mar está dominada principalmente por ligandos orgánicos, como la materia orgánica disuelta (DOM). El presente estudio tiene como objetivo estudiar la especiación química del Cu en una laguna costera, afectada por la minería y la eutrofización, y que presenta altos niveles de Cu y DOC. La especiación química del cobre (Cu) disuelto se estudió mediante dos métodos voltamétricos diferentes conocidos como voltamperometría de stripping catódico (CLE-ACSV), utilizando salicilaldoxima (SA) como ligando competidor, y voltamperometría de stripping anódico (ASV). Se tomaron muestras en diferentes localizaciones de la laguna del Mar Menor en diferentes eventos de 2021 y 2022. Los parámetros de especiación de Cu (capacidad de complejación y constante de estabilidad condicional) se calcularon mediante el software ProMCC, y los valores obtenidos para las diferentes muestras se relacionaron con variables fisicoquímicas. (pH, salinidad) y con diferentes variables relacionadas con la DOM como DOC, fluorescencia y absorbancia de la DOM, mediante análisis de correlación y regresión múltiple. Los resultados de la capacidad de complejación de Cu ( $L_{Cu}$ ) variaron de 15,89 a 90,82 nM para el método CLE-ACSV y de 58.28 a 193.5 para el método ASV. Además, los valores de la constante de estabilidad condicional ( $\log K_{Cu^{2+}}$ ) variaron entre 11.36 y 12.92 para el método CLE-ACSV y 8.195 y 9.609 para el método ASV. Los parámetros derivados de ambos métodos no se correlacionan entre ellos, lo que sugiere que estas técnicas podrían detectar diferentes clases de ligandos que tienen diferentes fuentes y comportamientos. Se observaron relaciones claras entre los parámetros de complejación obtenidos por CSV y DOC y los picos de fluorescencia relacionados con la materia húmica. Se considera que el uso de enfoques de ventanas múltiples que combinan los resultados de ambas técnicas describen completamente la complejación de Cu en muestras naturales.

### 3.11.P-Mo153 Understanding the influence of environmental factors on Co-magnetite interactions mechanisms in at the nanoscale

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Magnetite nanoparticles (MNs, Fe<sub>3</sub>O<sub>4</sub>) are abundant in the environment and are used for various applications due to their unique magnetic, adsorbing or redox properties. The stoichiometry of magnetite (Fe(II)/Fe(III)) is dependent on environmental factors (pH, presence of organic ligands), which largely affects MNs physico-chemical properties, such as adsorption of contaminants. However, the joint effects of environmental factors and MNs stoichiometry MNs-metal cations interaction are elusive. This study focusses on Co as an important contaminant in the environments, and because Co-doped MNs are of high interest for nanotechnology applications.

Magnetite nanoparticles (~10nm) with different stoichiometries were synthesized by co-precipitation, and partially oxidized using H<sub>2</sub>O<sub>2</sub> to obtain the desired stoichiometry. Batch studies were carried out with different [Co] (“adsorption isotherms”), using three different stoichiometries (0.1, 0.3 and 0.5), at different pH values and in the presence or the absence of natural organic matter (NOM). The electronic and magnetic properties of Co bound to MNs was probed by X-ray absorption spectroscopy (XAS) and magnetic circular dichroism (XMCD) at the Co L<sub>2,3</sub>-edges.

Experimental and modeling results revealed three Co species according to Co concentration, which could be attributed to (i) surface complexed or incorporated Co<sup>2+</sup> with a ferrimagnetic behavior at low loadings, (ii) magnetically-silent small Co polymers at intermediate loadings and (iii) precipitation of antiferromagnetic Co(OH)<sub>2(s)</sub>-like phase onto the MNs surface (i.e. core-shell Fe<sub>3</sub>O<sub>4</sub>@Co(OH)<sub>2</sub> nanoparticles) for highest Co concentrations. Their proportions depended on the physico-chemical conditions: Co-polymers and precipitates were favored on high-stoichiometry MNs, at high pH and in the absence of NOM.

This study provides detailed knowledge of the effects of environmental factors on the Co-MNs interaction mechanisms, and on the chemical and magnetic properties of these particles. This will help to predict the behavior and fate of Co in the environment and to understand the impact of environmental factors for an appropriate use of MNs for environmental applications.

### 3.11.P-Mo154 A Comparison Between a Simplified and an Extended Method for the Determination of Metals and Metalloids in Urban Park Soils

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The soil, together with the metals and metalloids present on it, can be incorporated into the human body by direct inhalation, ingestion and absorption by dermal contact. According to the recommendations of United States Environmental Protection Agency (USEPA), the daily ingestion values for soils are 200 mg·day<sup>-1</sup> for children from 1 to 12 years of age and 100 mg·day<sup>-1</sup> for people over 12 years of age. The bioaccessible fraction is defined as the fraction of the contaminant that is mobilised from soil into the digestive juice chyme and that fraction can be absorbed, transported through the intestinal wall and transferred to the bloodstream. As an alternative to in vivo experiments, it is possible to calculate the bioaccessible fraction through in vitro tests, such as SBET (Simplified Bioaccessibility Extraction Test) which simulates human gastric conditions and RIVM (Dutch National Institute for Public Health and Environment) which simulates mouth, gastric and intestinal conditions.

In this study, a total of 26 urban soil samples were collected in some parks of San Sebastian (Spain) and the bioaccessibility of some metals and metalloids was determined by SBET and RIVM methods. The main objectives of this study were to determine the bioaccessibility of the maximum number of metals and metalloids using two in vitro techniques and to compare the results obtained from both methods. Concentrations extracted with SBET method were generally above the concentrations extracted by RIVM. Cd was the most bioaccessible element in both methods, and the order from the most bioaccessible metal/metalloid to the least bioaccessible was quite similar in SBET and RIVM, except for Pb and Zn. Pb was identified as the second highest bioaccessible element by SBET (80.7%) and nearly the least bioaccessible by RIVM (0.9%), and the percentage of Zn was 34.7% by SBET and almost 0% by RIVM. The RSD of SBET triplicates was below 5% in almost all elements and LOD values were far from the metal concentration in SBET samples. In RIVM, RSD values were generally below 10% but for some metals (Zn, Ti, Cr) the LOD was quite high. Therefore, SBET resulted more economical but it can overestimate the risk on human health. On the other hand, RIVM is longer and more expensive, but it can provide a more realistic approach. However, in some elements, the high detection limit caused by the complexity of the matrix prevents the calculation of bioaccessibility by RIVM.

### 3.11.P-Mo155 Development of a predictive model for metal accumulation (Cu, Ni, and Zn) in periphytic biofilm

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In Canada, the mining sector is a major part of the economy but leads to an increase in metal mobility. The increase in metal concentrations in the environment can impact aquatic ecosystems and adequate monitoring is needed. Biofilm can be used as a

bioindicator for metal contamination and reflects actual metal bioavailability as opposed to simple measurements of metal concentrations in water. Bioavailability depends on many parameters such as pH, hardness, competition with other metals, and metal speciation. This study aims to develop a predictive model based on the premises of the Biotic Ligand Model (BLM) for copper, nickel and zinc accumulation in biofilm. Different environmental conditions were tested in the laboratory to calibrate the predictive model. We collected rocks with their biofilm in the Cap Rouge River (Quebec, Canada). This biofilm was placed in reconstituted water spiked with metals. A single parameter was modified at a time for each experiment to determine its influence on metal accumulation. A first experiment was completed in which a range of nine nickel concentrations were tested (from 0  $\mu\text{M}$  to 20  $\mu\text{M}$ ) at neutral pH and constant zinc and copper concentrations (0.1  $\mu\text{M}$ ). Similar experiments were carried out, varying the copper and zinc, while maintaining the concentrations of the other two metals constant (0.1  $\mu\text{M}$ ). As expected, metal accumulation increased in biofilm in proportion to the concentration present in the water. Additional experiments were conducted to test the protective effects of cations on biofilm accumulation. Copper, nickel and zinc concentrations were constant (10  $\mu\text{M}$ ) while pH, calcium and magnesium concentrations were individually varied in each experiment. Hardness cations are expected to compete with metals for accumulation in the biofilm; analyses are underway and results will be presented. This project will provide a predictive tool to estimate the impact of water quality on nickel, zinc and copper bioavailability.

### **3.11.P-Mo156 Metal(loid)s and Rare Earth Elements in *Posidonia oceanica* Banquettes along Mediterranean Coasts**

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The accumulation of *Posidonia oceanica* dead leaves occurs naturally along Mediterranean coasts constituting the so-called *banquettes*. They can provide important ecosystem services for beach nourishment and protection. Despite their important key role for energy transfer through marine-coastal habitats, *banquettes* are poorly investigated, especially concerning their double role as a contaminant accumulator and vector as well. Indeed, few studies exist in literature on the metal(loid)s occurrence and no data are available on emerging contaminants such as the Rare Earth Elements (REEs) in *Posidonia banquettes*. The present work investigated for the first time the concentrations of 28 metal(loid)s (Al, Sb, As, Ag, Ba, Be, Bi, B, Cd, Co, Cr, Fe, Li, Mn, Mo, Hg, Ni, Pb, Cu, Rb, Se, Sn, Sr, Tl, Te, U, V, and Zn) and 15 REEs (Ce, Dy, Er, Eu, Gd, Yb, Y, La, Lu, Nd, Ho, Pr, Sm, Tb, and Tm) in three well-structured *banquette* along the Mediterranean coasts: Lazio Region (Italy), Favignana Island (Sicily, Italy) and Cyprus. Sampling was carried out in 2021 (Lazio) and 2023 (Sicily, Cyprus).

Preliminary results showed (i) how metal(loid)s and REEs occurred in *Posidonia* deposits along eastern and western Mediterranean coasts, although values suggested no clear contamination patterns; (ii) that *Posidonia banquettes* may also represent an interesting biological model for monitoring metal(loid)s and REEs occurrence in coastal ecosystems.

### **3.11.P-Mo157 Characterizing monomethylmercury and selenium bioaccessibility from Alaskan fish and marine mammals**

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Mercury (Hg) is a global contaminant with known health risks to wildlife and humans. In Alaska (USA), humans are mostly exposed to Hg in the form of monomethylmercury through the consumption of fish and marine mammals, which compose large proportions of some traditional subsistence diets. Risk assessments of traditional Alaskan diets must weigh the cost of monomethylmercury exposure with the health benefits (e.g., nutrition, culture) associated with traditional foods. Risk assessors may assume that 100% of the monomethylmercury present in ingested food is released into the gastrointestinal fluid during digestion (i.e., is bioaccessible), despite growing evidence that this assumption is not accurate. In this study, we applied a dual-phase *in vitro* human gastrointestinal digestion model to characterize bioaccessibility of monomethylmercury found in muscle tissue of six fish species: burbot (*Lota lota*), Northern pike (*Esox lucius*), Pacific herring (*Clupea pallasii*), sockeye salmon (*Oncorhynchus nerka*), pink salmon (*Oncorhynchus gorbuscha*), and broad whitefish (*Coregonus nasus*) and two marine mammal species: walrus (*Odobenus rosmarus*) and Northern fur seal (*Callorhinus ursinus*). These species are commonly consumed in certain Alaskan communities, represent a range of trophic levels and muscle types, and include both freshwater and marine fish species. The monomethylmercury and total selenium concentrations in undigested muscle varied by species and ranged from 0.004  $\mu\text{g/g}$  to 0.71  $\mu\text{g/g}$  and 0.07  $\mu\text{g/g}$  to 5.9  $\mu\text{g/g}$  wet weight, respectively. The percent bioaccessibility of monomethylmercury varied significantly by species, with the highest seen in burbot (95.0  $\pm$  10.8%), followed by Pacific herring (80.0  $\pm$  10.0%), sockeye salmon (69.5  $\pm$  10.1%), pike (67.2  $\pm$  14.5%), Northern fur seal (45.2  $\pm$  4.5%), pink salmon (40.8  $\pm$  9.9%), walrus (38.5  $\pm$  6.4%) and the lowest seen in broad whitefish (34.3  $\pm$  8.6%). The bioaccessibility of total selenium will be characterized for these same fish and marine mammal samples, as selenium may protect against Hg toxicity. Our results indicate that less than 100% of the monomethylmercury present in the various muscle tissues is released during digestion, suggesting that current human exposures are being varyingly overestimated for these species and tissues.

### **3.11.P-Mo158 Kinetics of cobalt accumulation in soft tissues and shells of the gastropod *Radix balthica***

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Molluscs, including gastropods, are regularly used to assess the quality of natural waters through the use of their shells. Indeed,



shells have the ability to accumulate the chemical elements such as metals present in the mollusc environment throughout its life, while remaining inert. Shells should therefore serve as a bioarchive of metal contamination. Nevertheless, the link between the presence of metals in the environment and in shells is not always straightforward. The way in which individuals manage the accumulation of metals in soft bodies must play a role in this relationship. Here, we propose to study the link between metal concentration in water, soft tissue and shell in *Radix balthica*, a ubiquitous gastropod from European waters. The metal of interest was Co as pertinent substance to monitor in rivers.

To that end, *R. balthica* was exposed to four cobalt concentrations (natural background concentrations, 6, 30 and 60  $\mu\text{g}\cdot\text{L}^{-1}$  Co) during 28 days using outdoor mesocosms (TotalEnergies facility, France) filled with the Gave de Pau river water in June and October 2021. Water and organisms were collected after 7, 14, 21, 28 days of exposure for analysis. Water was analysed for their physico-chemical parameters including pH and cation concentrations. Total Co accumulation was measured soft tissues after acidic digestion whereas its subcellular distribution was examined using chemical treatment and differential centrifugation. Its concentration was also quantified in the whole shell after acidic digestion whereas its accumulation in the last shell growth stria was measured using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS).

Total Co accumulation in the soft tissues was already at equilibrium with the ambient water after 7 d of exposure for all Co concentrations tested. That accumulation was function of dissolved Co concentrations only for the highest exposure concentration. For example, Co accumulation in soft bodies increased 5.4-fold with the increase of Co exposure concentration from 6 to 60  $\mu\text{g}\cdot\text{L}^{-1}$  at 28 d. This equilibrium at 7 d was also observed with the whole shell and with the last growth shell stria. At 28 d, shell Co accumulation in the last growth deposit was measured to be  $0.97 \pm 0.47 \mu\text{g}\cdot\text{g}^{-1}$  and  $1.64 \pm 0.08 \mu\text{g}\cdot\text{g}^{-1}$  at 6 to 60  $\mu\text{g}\cdot\text{L}^{-1}$ , respectively. Future results on the subcellular Co distribution are expected to allow us to reconstruct Co pathway from water to the shell.

### 3.11.P-Mo159 Bioaccumulation of Selenium Oxyanions and Organoselenides in Stream Biota

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Selenium occurs in natural surface waters as a variety of inorganic and organic chemical species. The oxyanions selenate and selenite typically predominate. Organoselenide species, although hypothesized to be more bioavailable than the oxyanions, have rarely been identified or quantified in natural waters and little is known about their fate or bioaccumulative potential. We analyzed spatial patterns of bioaccumulation in relation to aqueous selenium speciation at more than 100 sites in southeast British Columbia, Canada. We used a sequential approach to fitting bioaccumulation model parameters, first using sites with no detectable organoselenium ( $<0.01 \mu\text{g}/\text{L}$ ) to describe the bioaccumulation of selenate and selenite, then applying those relationships to the remaining sites to infer the bioavailability of three detectable organoselenides. Our analysis indicated that the methylated organoselenide methylseleninic acid was the most bioaccumulative species, followed by dimethylselenoxide and methaneselenonic acid. Organoselenides were associated primarily with mine sediment ponds, and are presumed to be degradation products of selenium metabolism by algae and bacteria. Organoselenides exported from the ponds appear to be responsible for enhanced bioaccumulation in biota in downstream reaches. Our findings indicate that managing biological productivity in mine sediment ponds could help manage selenium risk in the receiving environment.

### 3.11.P-Mo160 Mining impacts on Chilean wildlife: heavy metals circulating in Andean fox blood

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Mining is one of the main activities of the Chilean economy, being the world's main producer of copper. *Las Tórtolas* is a tailings deposit with a 6,75 km<sup>2</sup> surface from *Los Bronces* copper mine located 40 km north of Santiago, where the Andean fox thrive in human-dominated landscapes. Wild carnivores are good sentinels that are able to reflect the environment they inhabit. The objective of this work was to analyse the levels of heavy metals in blood of foxes inhabiting mining habitats and assess the extent of exposure of these elements linked with adverse health effects. Blood analysis may provide useful information about the current exposure for antemortem assessment of heavy metals. A total of 42 Andean foxes (*Lycalopex culpaeus*) were sampled between May 2017 and May 2018 and a blood sample was obtained from each individual. Metal concentrations of 23 elements: vanadium (V), chrome (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), arsenic (As), selenium (Se), rubidium (Rb), strontium (Sr), molybdenum (Mo), silver (Ag), cadmium (Cd), antimony (Sb), cesium (Cs), barium (Ba), mercury (Hg), thallium (Tl), lead (Pb) and uranium (U)) were determined in lyophilized whole blood using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Some metals (Mn, Co, Ni, Cu, As, Rb, Cs) have been found in different concentration between areas, others (Cr, Fe, Zn, Ga, Mo), do not have no significant differences between zones but there are individuals with slightly outlier values. We did a Principal component analyses and found that the individuals grouped the same distribution the sampling zones have in the map. It seems that the area of sampling of the fox clearly determine their heavy metals concentrations in blood. The Cu concentrations were significantly higher in the core sites of the mining area but did not reach toxic levels for wildlife (less than 4  $\mu\text{g}/\text{g}$ ). The levels of all the metals will be investigated

and compared to already published works of other mining areas affecting wildlife. These results will proportionate data of the current status of heavy metals in wildlife from an important mining area of Chile and its health implication.

### 3.11.P-Mo161 Evaluation of Metals and Metalloids in Shellfish and Fish Obtained from Nigerian Markets

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Environmental pollution is currently a global challenge with metals and metalloids being predominant. They arise from both natural and anthropogenic sources and may accumulate in aquatic animals and various foodstuffs. Shellfish and fish are a veritable source of proteins, fats, vitamins, minerals, and other nutritional values. The contamination of aquatic foods by metals and metalloids could pose serious health dangers to humans, as, some of these metals and metalloids have been shown to be toxic. This study thus investigated the concentrations of the following metals Cadmium, Lead, Chromium, Mercury Nickel, Iron, Vanadium, Manganese and Magnesium in shellfish and fish obtained from African shops in the United Kingdom, The Republic of Ireland and from different markets across the Southern part of Nigeria using Atomic Absorption Spectrophotometer (AAS) and Inductively Coupled Plasma Mass Spectrometry (ICPMS). In the Catfish samples A, B and C, the concentrations levels of metals were as follows  $Cd < Pb < Cr < Ni < Mn < Zn < Fe$ . Pb, Ni were above the WHO/FAO permissible limits while the concentrations of Cr, Cd, Fe and Zn were below the WHO/FAO permissible limit. In 8 samples of fish and shell fish, which were obtained from different markets in 5 Southern states of Nigeria; periwinkles, Samples, periwinkles (*Tympanotonus fuscatus*), brackish water catfish (*Arius africanus*), Bonga fish (*Ethmalosa fimbriata*), prawns (*Macrobrachium vollehovenii*), Crayfish (*Palaemon hastatus*), and African catfish (*Clarias gariepinus*) the range of values of the metals and metalloids found were Cr: 0.10-2.04 mgkg<sup>-1</sup>, Pb: 0.100-0.68 mgkg<sup>-1</sup>, Mg: 1060-18600 mgkg<sup>-1</sup>, Hg: 0.030-0.298 mgkg<sup>-1</sup> Ni: 0.25-1.1 mgkg<sup>-1</sup> V: 0.20-3.7 mgkg<sup>-1</sup> and Cd: 0.005-2.08 mgkg<sup>-1</sup>. The Periwinkle samples had the highest values of five different metals and metalloids Mg, V, Cr, Ni, Pb, while the *Arius africanus* sample a marine catfish, recorded the highest concentration of Cd which is above the WHO/FAO permissible limit. The concentrations of Pb and Hg were above the WHO/FAO permissible limit in periwinkle and marine catfish samples respectively. The result from this study indicates the contamination of the aquatic foods from Nigeria by the metals investigated, the implications of human consumption of these shellfish and fish, may lead to adverse health conditions.

### 3.11.P-Mo162 Incorporation of Fe Oxides as an Additional Phase for Predicting Cadmium Toxicity in Oxidized Sediments

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Traditionally, metal toxicity in sediment has been assessed by measuring sulfide concentrations, assuming the predominant formation of insoluble metal sulfides (MeS) rendering them unavailable for uptake by benthic organisms. Building on this concept, the sediment biotic ligand model was developed involving both sulfide and organic carbon in sediment (*Environmental Toxicology & Chemistry*, 2005, 24, 2410), with an assumption that an excess of metals over sulfides will partition to organic carbon. This model holds validity in anoxic environments where sulfides and organic carbon play pivotal roles in metal binding. However, heavy metals sensitive to redox changes tend to be released from both MeS and organic carbon, for instance, in oxidized sediments. Literatures show increased concentrations of dissolved Cadmium under oxidizing conditions compared to reduced sediments. Such released metals will then be re-adsorbed onto Fe oxides, another major phase for metal binding. For improved cadmium toxicity prediction, we propose an advanced model that considers both Fe oxides and organic carbon contributions as well as sulfide in oxidized sediment. Partition coefficients ( $K_d$ ) for both phases were obtained using the Windermere Humic Aqueous Model, version 7 (UK Centre for Ecology and Hydrology, 2012), and the relationship with pH was derived by curve fitting to find the best fit for the data. Data obtained from previous studies show good agreement with the predicted  $K_d$  values. A comprehensive model equation incorporating these  $K_d$  values, Fe oxides, and organic carbon contents was derived. When compared to the experimental data from the sediment samples collected from 21 different regions in South Korea, the model demonstrates accurate predictions within one order of magnitude. This model serves as a valuable tool for predicting cadmium toxicity in an oxidized sediment.

### 3.11.P-Mo163 Effects of a chronic exposure to cobalt on primary producers and invertebrates in two microcosm studies

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Higher tier ecotoxicity tests that measure effects at the population and community levels are a means to assess the laboratory-to-field extrapolation of regulatory thresholds derived from single species laboratory tests. The primary objective of the current research was to validate a bioavailability normalised predicted no effect concentration (PNEC) for freshwater organisms which was derived from an SSD of chronic single species tests. Therefore, effects of constant exposure to cobalt on a freshwater community was monitored in two indoor microcosm studies. A further aim of the studies was to provide information on the fate of cobalt in freshwater ecosystems and especially on the accumulation in biota. The first study was a 56d fully replicated study, while the second was an 84d regression-based study that was conducted in larger tanks and which considered additional community level endpoints. Both studies included taxa that were identified as sensitive to chronic cobalt exposure in single-species laboratory tests, i.e., primary producers and invertebrates. The accumulation of cobalt was assessed in a sub-set of organisms - periphyton, macrophytes, snails – with the intention of providing accumulation data at different trophic levels, and food-chain transfer of cobalt from primary producers to a grazer consumer. Overall, the two microcosm studies provide

population effect levels for several groups of organisms - phytoplankton, periphyton, macrophytes, zooplankton, macroinvertebrates – as well as effect levels for ecosystem functions, such as leaf litter degradation. Results are therefore valid to inform on the protectiveness of a cobalt PNEC derived from the SSD.

### **3.11.P-Mo164 Speciation and Ecotoxicity of the Soluble Fraction of Tellurium and Tellurium Dioxide**

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Following an ECHA decision for the provision of additional long-term ecotoxicological information for the sparingly soluble substances Tellurium metal (EC 236-813-4) and Tellurium dioxide (EC 231-193-1), a scientific research program was initiated by the REACH Selenium and Tellurium Consortium<sup>3</sup> to meet the new data requirements. Since the Water-Accommodated Fraction approach cannot be applied on sparingly soluble inorganic substances, the identified data gaps could only be filled by read-across from data generated with a soluble Te-salt.

In a first step, 24h-screening T/Dp tests were conducted with both substances at different pH-levels for the identification of the Te-speciation of the soluble fraction. Similar to other metals, Te can exist in various oxidation states, and toxicity among these oxidation states may vary. Information on the speciation profile of the soluble Te- and TeO<sub>2</sub>-fraction is therefore essential for selecting the appropriate soluble Te-salt(s) to be used in the testing program. Analysis of the T/Dp solution, performed at the Flemish Institute for Technological Research (VITO), showed that more than 99% of dissolved Te from either Te-metal or TeO<sub>2</sub> was present as Te(IV), and thus Na<sub>2</sub>TeO<sub>3</sub> was selected as the read-across soluble test substance .

Long-term toxicity OECD Guideline studies were conducted with algae, cladocerans and fish (ELS-test), and revealed that *Daphnia magna* was the most sensitive species (21d-EC<sub>10</sub> of 72 µg Te/L). The high sensitivity of *D. magna* towards Te was previously observed in short-term testing with these three species.

The concentration of 72 µg Te/L represents the chronic ecotoxicity reference value that can be used for both classification purposes and revision of the current PNEC. Chronic classifications are determined by comparing the ERV with 28d T/Dp data that were previously determined.

The revised Te-PNEC is 7.2 µg Te/L (AF of 10 on the lowest chronic value), a value that is slightly higher than the original PNEC of 5.79 µg Te/L which was determined by applying an AF of 1000 on the lowest acute value (5.79 mg Te/L for *D. magna*). Taking the molecular weight of TeO<sub>2</sub> into account, a PNEC of 9.0 µg TeO<sub>2</sub>/L is also calculated. Both PNEC-values are considered relevant as they are lower than the maximum solubility of these two substances.

### **3.11.P-Mo165 The DOM-inating problem– Assessing the impact of organic matter chelation on mercury toxicity**

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Current ecotoxicological testing demands full standardisation for established protocols to ensure inter-laboratory comparability as the choice and exact composition of exposure media can have a significant influence on toxicant chemistry and animal welfare. Subsequent data production and interpretation is thus subject to a deep understanding of the underlying environmental chemistry and consideration of organismal health.

Mercury is a potent neurotoxin and listed on the top ten of chemicals of major public health concern by the WHO. Its unique chemical properties enable mercury to speciate and thus change its environmental behaviour based on its surrounding. One of the key elements influencing the environmental fate of mercury is organic matter, due to its abundance of nonspecific functional groups and chelating properties. The impact of organic matter on the chemical behaviour of mercury has been demonstrated for terrestrial and freshwater systems, while a similar quantification for marine environments remains fragmentary. However, evaluating the impact of organic matter-mercury interactions at low trophic levels is important as they serve as springboards to complex environmental food webs.

We used the euryhaline crustacean *Artemia salina* to assess the bioaccumulation and toxicity of two mercury compounds in saline media with contrasting concentrations and sources of dissolved organic matter. The toxicity to cysts and hatched individuals was assessed in 24h assays, and their bioaccumulation rates determined using empirical uptake analysis and single-compartment toxicokinetic modelling. Additionally, mercury nanoparticles were employed for the first time in an acute toxicity study.

Our results suggest that media composition plays a pivotal role in marine aquatic toxicology, thus emphasising the importance of standardising the underlying procedures. Dissolved organic matter significantly impacts bioaccumulation rates as well as acute toxicity and excystment. The study emphasises the need for a standardised seawater recipe with full control of all variables to elevate marine ecotoxicological testing.

### **3.11.P-Mo166 A Holistic Modelling Approach to Predict Silver (Ag) Toxicity on Rainbow Trout Populations**

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Metal bioavailability plays a pivotal role in determining the toxicity of silver (Ag) to aquatic fish species, such as the rainbow trout (*Oncorhynchus mykiss*). Ag exposure disrupts sodium (Na) uptake at the gills, leading to perturbations in the internal Na-balance, which results in elevated blood viscosity, cardiovascular failure, and mortality. This study builds upon the existing sodium balance model (SBM) to mechanistically describe the impact of Ag on Na-concentration within rainbow trout, thereby predicting lethal outcomes under specific exposure conditions. However, understanding the broader ecological implications of Ag toxicity and its differential effects across various life stages remain underexplored. This research aims to bridge this knowledge gap by integrating biotic ligand models (BLMs), SBM, and individual-based modelling (IBM) to assess the realistic effects of Ag on rainbow trout populations.

To achieve our objectives, we combined the BLM and SBM with an IBM, incorporating individual threshold (IT) and stochastic death (SD) approaches to simulate lethal Ag effects. We based our environmental bioavailability conditions on the FOREGS dataset to ensure realism in our predictions. The results of our integrated modelling framework highlight the significant impact of Ag exposure on rainbow trout populations, particularly when the juvenile life stages are affected. At environmentally relevant Ag concentrations, the SBM did not predict any effects under realistic bioavailability (physico-chemical) conditions. Integrating SBM with an IBM approach allowed us to extrapolate these effects to the population level, ultimately deriving population-level thresholds. Our findings reveal that the juvenile life stage appears to be the most sensitive and crucial in determining population effects, with significant implications for regulatory testing strategies.

Our modelling framework incorporates various modelling approaches (including General Unified Threshold model for Survival [GUTS], BLM, SBM, and IBM) while combining exposure and effect data, to provide predictions on Ag toxicity to rainbow trout populations. Such holistic approaches are instrumental for making informed decisions regarding further testing strategies for Ag toxicity. This research serves also as a valuable step toward developing risk assessment strategies to protect fish populations in European freshwater environments.

### **3.11.P-Mo167 Environmentally Relevant Toxicity Assessment of Metal Mixtures for the Egg and Sac-fry Stages of Japanese Medaka and the Reproduction of *Ceriodaphnia dubia***

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To conduct risk assessments of metal mixtures that are environmentally relevant by regulatory agencies, it is essential to predict the ecotoxicological effects of these mixtures on organisms under realistic exposure profiles. In this study, we examined whether the short-term chronic toxicity of metal mixtures, reflecting the combinations and concentration ratios of metals found in Japanese rivers, could be predicted for Japanese Medaka and *Ceriodaphnia dubia*. This prediction was based on the results of toxicity tests of individual metals using the two species. We conducted short-term and chronic toxicity tests of five metals (Al, Cd, Cu, Ni and Zn) on egg and sac-fry stages of Japanese Medaka. We also collected data on toxicity tests of *C. dubia* reproduction for these metals from published articles. Subsequently, we conducted toxicity tests on metal mixtures using the two species, reflecting exposure profiles that are environmentally relevant in Japanese rivers. Metal speciation in test solutions was determined using a chemical speciation model and/or measured by the DGT filter method to estimate bioavailability-based toxicity of individual metals and metal mixtures. The bioavailability-based effects of metal mixtures on the egg and sac-fry stages of Japanese Medaka and the reproduction of *C. dubia* were then compared with estimates from results of toxicity tests of single metals based on concentration additive and independent action models. The methods for predicting the toxicity of metal mixtures from data on toxicity tests of single metals in Japanese rivers will be discussed based on results.

### **3.11.P-Mo168 Effects Of Essential And Non-Essential Metals In The Photosynthetic Parameters Of A Freshwater Chlorophyceae**

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Algae are at the base of the trophic chain and can impact higher trophic levels. Some metals are required for the optimal metabolism of the algae, while others are not required for metabolic processes. Independent of the essentiality, higher concentrations of metals are toxic to different trophic levels. Cobalt (Co) is an essential metal, being part of enzymes and B12 vitamin. Chromium (Cr), on the other hand, has no known functions for algae. Cr is common and persistent in the environment, being present in the forms  $Cr^{2+}$ ,  $Cr^{3+}$ , and  $Cr^{6+}$ , with different toxicities.  $Cr^{3+}$ , used in the present study, has low membrane permeability and low capacity of bioaccumulation in the trophic chain. In the present study, cells of the freshwater microalga *Ankistrodesmus densus* (Chlorophyceae) in exponential growth phase were exposed to three concentrations of Co and Cr: 0.2, 0.4 and 0.8 mg L<sup>-1</sup>. The photosynthetic activity of microalgae (maximum and effective quantum yields, photochemical and non-photochemical quenching, and rapid light curves) was assessed using pulse amplitude modulated fluorometer (Phyto-PAM) and parameters of dark- and light-adapted samples were measured after 72 h. Under metal exposure, the relative maximum electron transport rate was the most sensitive parameter (decrease 66-70%). However, for the other evaluated parameters our data indicate that all parameters were affected in the presence of metals, with a higher sensitivity in the photosynthetic parameters of algae exposed to Co. In addition, algae was efficient in activating photoprotective mechanisms, avoiding damages in the photosynthetic machinery. Although, it is not possible to infer with certainty if the higher toxicity of Co can be related to its essentiality, which could result in higher uptake by the cells; or due to the different

charges of both metals, i.e.,  $\text{Co}^{2+}$  could compete with  $\text{Mg}^{2+}$  or  $\text{Mn}^{2+}$ , affecting chlorophyll and oxygen evolving complex, respectively.

### 3.11.P-Mo169 Effects of copper on the fatty acids' profile of standard freshwater species

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The waterborne copper concentration has been increasing over the decades mainly associated with industrial and agricultural inputs. Remarkably, this metal is one of the most extensively used agrochemicals (both as fertilizer or pesticide) in Europe, employed for the elimination or control of fungi, bacteria, and algae. While copper can be physiologically essential at low concentrations, its toxicity becomes apparent at elevated levels, impacting organisms across the spectrum from primary producers to secondary consumers. Consequently, once introduced into water, copper exhibits persistence and can induce adverse effects on diverse aquatic organisms. Thus, it is crucial to determine lethal and sub-lethal (e.g., biochemical responses) effects of aquatic organisms to predict the impacts of copper on ecosystem's health. Fatty acids (FAs) are molecules that play an essential role in the maintenance of physiological functions of many organisms being for example the major constituent of the cell wall as part of phospholipids. Furthermore, alterations in FAs profile can act as early warning signals of stressing conditions becoming an effective ecotoxicological tool. Hence, this study aimed to assess the effects of sub-lethal copper concentrations on the FAs profile of standard freshwater species (*Raphidocelis subcapitata*, *Lemna minor*, *Daphnia magna* and *Chironomus riparius*). Firstly, the copper  $\text{EC}_x$  for all species was determined subjecting the organisms to a range of non-lethal to lethal copper concentrations. Considering the values obtained for each species, biochemical bioassays were performed using a concentration below  $\text{EC}_{10}$  and equal to  $\text{EC}_{10}$  and  $\text{EC}_{20}$ , respectively. Results pointed out that *D. magna* is the most sensitive planktonic species to copper, followed by *L. minor* and *R. subcapitata*. Moreover, in this study, the species from different trophic levels exposed to a range of copper concentrations revealed changes in its fatty acid content. There was an increasing of saturated FAs (mainly composed by Palmitic acid) and polyunsaturated FAs (mainly composed by the essential fatty acid  $\alpha$ -Linolenic acid – ALA – omega-3, with exception of *C. riparius*) with increasing of copper concentrations, for all species with exception of *R. subcapitata*. FAs proved to be a useful bio-indicator of the healthy status of aquatic ecosystems, providing crucial information about the impacts of copper in the trophic food web.

### 3.11.P-Mo170 Associations of Heavy Metal Exposure with Dyslipidemia and Elevated Liver Enzymes

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Lead and Mercury has permanent adverse effects on human and this exposure has become one of the major public health problems. The purpose of this study was to investigate the association of blood levels of lead(Pb) and mercury(Hg) with dyslipidemia and liver function. Analyses were performed with a total of 2,952 participants in the KNEHS(Korea National Environmental Health Survey) from 2018 to 2020. KNEHS is a cross-sectional study that has calculated national statistical data on the level of exposure to environmentally harmful substances in the body since 2009. The blood lead levels were significantly higher in the dyslipidemia group (n=931, 1.609ug/dL) compared to non-dyslipidemia (n=2014, 1.456ug/dL). Similarly, those with elevated liver enzymes (n=744, 1.780ug/dL) exhibited higher lead levels than the normal group (n=2201, 1.415ug/dL). The blood mercury levels also showed significant elevation in the dyslipidemia group (3.263ug/L vs.2.809ug/L) and elevated liver enzymes group (3.815ug/L vs.2.686ug/L) compared to the control group. In logistic regression analysis, dyslipidemia was significantly associated with blood lead (OR 1.609; 95% CI: 1.243-2.084) and mercury level (OR 1.403; 95% CI: 1.178-1.672). Elevated liver enzymes were similarly associated with blood lead (OR 3.148; 95% CI: 2.350-4.217) and mercury level (OR 2.272; 95% CI: 1.869-2.762). This study showed a strong link between blood levels of lead and mercury, dyslipidemias, and elevated liver enzymes, emphasizing the potential health risks posed by exposure to these heavy metals.

### 3.11.P-Mo171 Kinetics of Meta-metabolome Response to Cobalt Exposure in Dynamic River Biofilm Communities

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As part of the energy transition away from fossil fuels, the extraction and use of cobalt (Co) has increased significantly in recent decades. Although few studies have investigated its toxicity, it is currently classified as a substance to monitor in rivers in some countries. To provide Co toxicity data needed to assess the ecological risk for aquatic organisms, mature biofilms were

exposed for one month in artificial river streams to four Co concentrations (background concentration,  $1 \times 10^{-7}$ ,  $5 \times 10^{-7}$  and  $1 \times 10^{-6}$  M). Bioaccumulation, biomass, chlorophyll-*a* levels and the composition and structure of prokaryotic and eukaryotic communities were monitored along with the meta-metabolomic response at different time points (from 1 hour to 28 days). Using an untargeted metabolomic approach, the entire biofilm meta-metabolome was characterized using a new holistic data processing approach based on dose-response modeling, benchmark-dose/time calculation and characterization of response trends. From these results, two ranges of concentrations were observed at each time step. The first range was characterized as a concentration range inducing defense responses (CRIDeR) and the second as a concentration range inducing damage responses (CRIDaR). The initial thresholds of CRIDeR were found to be similar between each exposure time, whereas those of CRIDaR increased as a function of exposure time. This result suggested a community shift toward tolerant species, which was confirmed with the characterization of the prokaryotic and eukaryotic communities. Moreover, we also demonstrated that changes in the meta-metabolome were faster than changes in community composition using a Multivariate Omics Trajectory Analysis. These rapid changes in the meta-metabolome were confirmed by the observation of time ranges inducing defense and damage responses (TRIDeR and TRIDaR) based also on the interpretation of trends in dose-response models of untargeted meta-metabolomic features as a function of exposure time. The initial thresholds of TRIDeR were measured to be a few seconds whereas those of TRIDaR were in the order of 1 day. This study demonstrates that meta-metabolome defenses and damage responses of Co-exposed biofilms are rapidly and continuously impaired, even within resistant and tolerant microbial communities.

### 3.11.P-Mo172 Accumulation and Effects of Cobalt on Microbial Communities Living in Growing Freshwater Biofilms.

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Cobalt (Co) is one of the metals whose use and extraction have increased considerably over the last few decades. As it is likely to find its way into the environment in the near future, it is vital to assess its impact now. Although they are not widely used in chemical risk assessment, prokaryotic and eukaryotic microorganisms play a vital role in the proper functioning of ecosystems. They can develop in the form of biofilms on submerged substrates in rivers. Freshwater biofilms are ubiquitous and dynamic biological systems harboring a wide diversity of microorganisms involved in the cycling of essential elements. Directly exposed to contamination induced by human activities in freshwater, biofilms are indicators of the health of aquatic ecosystems. This study aimed at deciphering how cobalt impacts the biological organization and functioning of aquatic biofilms.

To that end, artificial substrata were placed in mesocosms (TotalEnergies facilities, Pau, France) contaminated with Co (0.1, 0.5 and 1  $\mu$ M) or containing background Co concentrations (control) to colonize biofilms for 28 days. After the exposure, a period of 5 weeks was further applied without Co contamination to simulate a recovery period. Water and biofilm samples were collected every 7 days during Co exposure. Water was analyzed for its water quality, including Co concentrations. Biofilms were examined for Co bioaccumulation, and their structure and functional potential.

Cobalt bioaccumulation in biofilms was mainly intracellular and well correlated with the concentrations tested. The presence of Co at concentrations of 0.5 and 1  $\mu$ M increased the abundance of prokaryotes from the 21<sup>st</sup> day of exposure and their diversity was also impacted since the early stages of colonization. Indeed, *Cyanobacteria* were highly sensitive and not able to colonize the biofilm support from day 7 for concentrations above 0.1  $\mu$ M. In contrast, *Bacteroidota* appeared resistant all through the biofilm colonization. Microeukaryotic abundance was lower at day 21 for 0.5 and 1  $\mu$ M of Co as compared to the control, which was accompanied with a change in beta-diversity for exposed biofilms since first days of colonization. Co-occurrence networks between prokaryotic and microeukaryotic communities are under analysis. Finally, functional potential analyses based on prokaryotic communities' composition revealed that Co influenced a number of cellular processes and primary production, a determining process in the biofilm formation.

### 3.11.P-Mo173 Temporal and Spatial Changes in Water Quality and Phytoplankton Populations in the lower St. Johns River, Florida

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The St. Johns River (SJR) is an ecologically and economically important estuarine river system in Florida undergoing extensive anthropogenic change. In this study, a variety of water quality parameters and a suite of metals were measured in water samples collected from eight sites in the lower SJR, in Florida from 2019 to 2022, continuing from previous work that documented these parameters in the river from 2017 to 2019. Aquatic communities such as phytoplankton can be indicative of river health; therefore, phytoplankton were collected from each site, and the diatom component was identified. The total number of taxa in each sample ranged from 60 to 190, with 25 taxa accounting for the majority (64%). Similar to water chemistry, seasonal fluctuations in phytoplankton abundance and diversity were observed, with a relative abundance of *Skeletonema costatum* and *Skeletonema subsalsum* in times of lowered diversity. Furthermore, decreased phytoplankton diversity correlated with increased metal contamination in the lower SJR. Multivariate analyses highlighted significant

interactions among phytoplankton diversity and water chemistry variables. This study provides new information about the impact of human disturbance on biotic communities.

### **3.11.P-Mo174 Does Cryptic Diversity Within the *Eurytemora affinis* Complex Show Differential Sensitivity to Lithium Exposure?**

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A cryptic species complex encompasses morphologically indistinguishable species that are genetically divergent. Some of the most frequently used species in ecotoxicological assessment show evidence of cryptic diversity that may affect the results of a given test. Linking cryptic diversity to sensitivity of chemicals has rarely been investigated. Lithium (Li) is an alkali metal considered as one of the most important emerging pollutants under concern due to high demand for this compound essentially to produce rechargeable batteries. Li environmental concentrations are expected to increase in the next years and its effects on marine and coastal organisms remain relatively unrecognized. Here, we focused on two of six cryptic species of the estuarine copepod complex *Eurytemora affinis*, exposing natural populations on two continents. Our main objective was to characterize the differential sensitivity of these two-field collected *E. affinis* cryptic species (the European and the North-Atlantic clade) to Li using a larval bioassay. Oviparous females of the North-Atlantic clade were sampled in the St. Lawrence estuary (Canada) and for the European clade in the Seine estuary (France) and incubated to recover early-stage larvae (nauplii). We assessed survival and growth of *E. affinis* nauplii exposed to a gradient of Li concentrations (1, 10, 50 and 100 mg.L<sup>-1</sup>) for 96 h at 5 PSU and 15°C. LC50 were estimated by using log-logistic model. LC50 for the nauplii from North-Atlantic and European clades of *E. affinis* were 243.42 (± 3.56) mg.L<sup>-1</sup> and 241.41 (± 2.05) mg.L<sup>-1</sup> at 24 h exposure and 86.31 (± 1.07) mg.L<sup>-1</sup> and 72.78 (± 1.15) mg.L<sup>-1</sup> respectively after 96 h. Li inhibit growth rate of nauplii at ≥ 50 mg.L<sup>-1</sup> with an EC50 at 96 h of 60.19 (± 1.13) mg.L<sup>-1</sup> for the North-Atlantic clade nauplii. The estimated EC/LC50 values were too high to cause extreme effects at environmental concentrations observed in Northern Hemisphere estuaries. No significant differences were observed in Li sensitivity between the two clades. However, further studies should be performed to test other contaminants, to improve knowledge on sensitivity of the cryptic species *E. affinis* complex to assure routine ecotoxicological tests are appropriate.

### **3.11.P-Mo175 Challenges of Deriving Environmental Quality Standards for Cerium and Lanthanum**

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Lanthanide compounds, specifically Cerium (Ce) and Lanthanum (La) salts, are relatively new alternatives to other commonly used flocculants in water treatment plants. While their use provides some practical advantages, the impact on aquatic environment also needs to be evaluated. We derived environmental quality standards (EQSs) based on the EU Technical Guidance document No. 27 for EQS derivation under the Water Framework Directive (WFD). This guidance is also used in Switzerland.

Aquatic toxicity, environmental fate and physical-chemical properties data were collected from all publicly available resources and assessed for relevance and reliability according to the CRED (Criteria for Reporting and Evaluating ecotoxicity Data) method.

For La, 101 acute, 50 chronic and 3 sub-chronic effect values were evaluated. For Ce, the evaluation included 50 acute, 17 chronic and 2 sub-chronic effect values. As speciation and bioavailability of Ce and La vary under various physical-chemical conditions, only effect studies reporting measured dissolved concentrations and effects related to these were considered.

Insufficient documentation of scientific studies in terms of specification of experimental and analytical conditions led to the exclusion of most studies from open literature. The scientific and technical issues narrowed down the usable data almost exclusively to studies submitted for REACH registration purposes. However, studies from the database of the European Chemical Agency (ECHA) could not be validated, as the reliability of the presented data is unclear in view of missing and contradictory information. The study summaries are often incomplete and neither the official assessment nor the original study reports are available publicly. In the absence of original study reports, the results could not be verified and only preliminary EQSs could be derived that are in the range of measured background concentrations of Ce and La.

As Ce and La occur naturally in the environment, instead of absolute EQS values, we would recommend the definition of maximum permissible additions (MPA) to the local background concentrations as already in place in the Netherlands.

Improvement towards a more robust scientific outcome can be achieved through analytical verification of exposure and complete documentation of experimental studies as well as better managed databases with access to official assessments and original study reports.

### **3.11.P-Mo176 Evaluating the Sensitivity of Environmental Threshold Derivation of Cu in Europe to the Use of Geographically Relevant Species**

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Chronic toxicity of Cu is dependent on the physico-chemistry of freshwater environments. More specifically, pH, DOC, Ca, Na and Mg have been shown to influence chronic toxicity to aquatic organisms. Hence, the derivation of environmental thresholds of Cu for the freshwater environment in Europe consider the influence of local conditions using bioavailability models. The derivation of environmental thresholds for Cu in Europe, such as the environmental quality standard, is currently under review. These ongoing revisions consider updates to the chronic Cu toxicity database, and of the bioavailability models. Toxicity databases of many metals are typically data-rich, i.e., they include reliable chronic freshwater data of numerous species. For Cu, high-quality data have been identified for 44 freshwater species and covering 336 individual data-entries. Species sensitivity distribution (SSD) approaches used in the environmental threshold derivation assume that the species in the Cu database represent surrogates for species occurring in natural European ecosystems. The use of species that are not representative for the ecosystem to be protected has previously been criticized in literature, as these may not represent the sensitivity of local species. Some jurisdictions, e.g., Australia, consider the geographical relevance of a species for a specific region as a criterion to collate SSDs.

The Cu freshwater database contains 16 species that occur in Europe. Next to this group, ‘European relevant species’ consider also those non-European species that have a close relative (at the genus-level) occurring in Europe, but for which the genus is not yet represented among the European species in the database (5 in total). The remaining species include several species belonging to genera and/or families that are endemic to non-European regions. In the present study, we evaluate several scenarios that assess the influence of considering only ‘European relevant species’ on the environmental threshold derivation for Cu. This will be done by combining the most recent bioavailability normalization approaches with SSD-techniques (using the Cu bioavailability tool CuBioM) using physico-chemistry databases relevant for European freshwaters. Our testing hypothesis is that the consideration of European relevant species has limited influence on environmental threshold derivation. Our study may improve the geographical relevance of risk and compliance assessments for Cu in Europe.

### **3.11.P-Mo177 Introducing the geographical relevancy of species to set environmental thresholds of Pb for aquatic environments in Europe**

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Chronic toxicity of Pb is strongly dependent on the physico-chemistry of freshwater environments. More specifically, DOC and pH have been previously shown to influence the chronic toxicity of Pb to freshwater organisms. As such, bioavailability models have been developed to account for the influence of these toxicity modifying factors. Hence, the derivation of environmental thresholds of Pb for the freshwater environment in Europe and many other regions is bioavailability-based. For the marine environment, no specific bioavailability correction has been introduced. Recently, chronic Pb toxicity databases have been updated with published chronic toxicity data for both freshwater and marine species. High-quality toxicity data have been identified for 34 freshwater species covering 191 individual data-entries, and for 36 marine species covering 85 individual data-entries. The species sensitivity distribution (SSD) approach has been used to derive the environmental threshold as it assumes that the species in the Pb database represent surrogates for species occurring in natural European ecosystem. Surrogate species may or may not represent the sensitivity of populations of local species. The use of species that are not representative for the ecosystem to be protected has previously been criticized in literature. The Pb toxicity database contains 26 species that are representative for European freshwaters, and 27 species for European marine waters. In the present study, we evaluate several scenarios that assess the influence of considering only ‘European relevant species’ on the environmental threshold for Pb, by combining the bioavailability normalization approaches with SSD-techniques using physico-chemistry databases relevant for European freshwaters. Consistent to our testing hypothesis, the results reveal that the consideration of European relevant species, which increases the robustness of the assessment, has limited influence on Pb environmental thresholds for freshwater and marine environments.

### **3.11.P-Mo178 EU-WIDE EXPOSURE ASSESSMENT OF METALS IN MUNICIPAL SEWAGE TREATMENT PLANTS**

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Releases from consumer and professional widespread uses to the aquatic environment are based on default assumptions leading to precautionary assessments in the REACH dossiers. Around 80-90% of urban wastewater is collected and treated in the EU. Hence metal emissions from e.g. consumer use of products via STP effluents are not to be neglected. Emissions from municipal sewage treatment plants (STP) can provide a better estimate, given they collect metal emissions from these combined sources.

Providing a realistic estimate of metal concentrations in the effluent of municipal STPs is part of Eurometaux’s ongoing comprehensive “Metals Environmental Exposure Data gathering” (MEED), covering today’s and tomorrow’s need to comply with the REACH combined effects, Zero Pollution Ambition and biodiversity objectives.



The EU's Urban Wastewater Treatment Directive sets requirements for urban wastewater quality but lacks specific regulations for monitoring metals. As a result, there is no centralized database for metal concentration data in the EU. To address this gap and collect data, environmental and water agencies across EU countries were contacted for metal emission data.

Data sets for 20 metals, covering up to 12 countries (availability of data varied between countries), were compiled, and represented the basis for the derivation of reasonable worst-case STP effluent concentration.

The methodology included an outlier analysis to minimize the impact of extreme local point sources (e.g. due to contamination with small industrial sources) when deriving a representative STP effluent concentration.

The EU  $PEC_{STP}$  was represented by the median of all 90<sup>th</sup> percentile values from the different countries. The comparison of the EU  $PEC_{STP}$  value with the  $PNEC_{STP}$  was made to derive the risk characterization ratios (RCR) at EU scale.

All RCRs for the individual metals were below 1 for the STP. The RCRs for the subsequent environmental compartments after STP discharge were all below 1 except for two metals with RCRs > 1 for the sediment compartment. When also including the regional background concentrations, a significant portion of the RCRs are larger than 0.2 hence will require an assessment of the combined risk in the EU.

Nonetheless, this does not imply the existence of an actual risk. These values could potentially undergo further refinement by incorporating the bioavailability concept, although such refinement lies beyond the scope of this project.

### **3.11.P-Mo179 REGIONAL EXPOSURE ASSESSMENT OF METALS IN THE AQUATIC FRESHWATER ENVIRONMENT – SEDIMENT COMPARTMENT**

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Environmental release rates of metals can vary over time due to new uses and changes in used/manufacturer volumes, but also due to improvement of emission-reducing technologies and external conditions like climate change. As the sediment compartment serves as the primary environmental sink for most metals, changes in metal emissions to water could have a significant impact. Consequently, previously collected regional ambient concentrations used in a regulatory context (e.g. for exposure scenario development under REACH) may not be relevant anymore for current or future situations. An update of these regional metal concentrations in the sediment compartment is part of Eurometaux's currently ongoing comprehensive "Metals Environmental Exposure Data" (MEED) program, covering today's and tomorrow's expected needs to comply with the REACH combined exposure, the Zero Pollution Ambition and biodiversity objectives.

Most countries do not measure metal concentrations in sediment as part of their regulatory-based monitoring program and, additionally, there is no unified European Union (EU) monitoring database.

The absence of such datasets required a customized approach, involving data collection from diverse sources such as national websites and personal communication.

Data sets for 28 metals from 6 databases were compiled to derive the EU  $PEC_{regional}$  sediment value according to EU Guidance Documents. To address inherent biases within the datasets due to increased local monitoring efforts at highly historically contaminated sites, the EU  $PEC_{regional}$  was calculated based on median concentrations.

When comparing the revised EU  $PEC_{regional}$  values with the existing values used for exposure modelling purposes, the revised values are generally lower. However this is mainly due to the slightly different methodology. However, it is worth noting that Co, V, and Zn were clear exceptions hinting towards increasing concentrations. The trend assessment was only possible for metals with previously assessed EU  $PEC_{regional}$  values, namely: Ag, Co, Cu, Mo, Ni, Pb, Sb, V and Zn.

In conclusion, the project successfully updated the EU  $PEC_{regional}$  sediment values for a large number of metals by utilizing multiple databases and applying a standardized approach to ensure consistency. The revised EU  $PEC_{regional}$  values based on monitoring data provide more accurate estimates for risk assessments and exposure modelling to help measuring progress to EU environmental quality ambitions.

### **3.11.P-Mo180 Updating The European Freshwater Annual Average EQS For Nickel: What Are The Environmental Consequences?**

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Under the European Water Framework Directive (WFD) Environmental Quality Standards (EQS) for nickel (Ni) have been set to be applied across all of Europe. In 2013, an annual average EQS for nickel of  $4 \mu\text{g L}^{-1}$  was derived to account for bioavailability. However, since this time new chronic ecotoxicity data have become available; in 2013 31 species, and 214 data

points, to 56 species and 366 data points in 2023. Furthermore, bioavailability models (Biotic Ligand Models, BLMs) have been updated covering a much greater range of water chemistry conditions (in Europe this increases waters covered from 88% to >98%).

In this study we consider the implications of updating the EQS in line with the best currently available science and European Guidance, using the UK as an example, to evaluate whether there are any important differences between the existing Ni EQS and what would be derived based on the best currently available science.

The key findings of the study are that increasing water chemistry coverage with the new bioavailability-models means including more waters that are sensitive to nickel exposures. A revised Ni EQS in the UK would be  $3.1 \mu\text{g L}^{-1}$ , which would be protective of 95% of waters in North West Region (of England), which is a reduction from the current value for the EQS of  $4 \mu\text{g L}^{-1}$ . The European Commission has recently proposed to amend the Ni EQS to a value of  $2 \mu\text{g L}^{-1}$ . Unfortunately, this amended value does not include the ecotoxicity dataset for nickel produced since 2013 (so does not include the new tested species), nor the new BLMs (so does not increase water chemistry coverage), and simply applies an assessment factor of 2 to the existing AA EQS of  $4 \mu\text{g L}^{-1}$ .

Utilizing the best available science, including datasets, models and concepts, and following the existing guidance are imperative for the successful derivation and implementation of metal EQS.

### **3.11.P-Mo181 Comparison of Copper Dust Exposure Across Multiple Operations in Europe that Process Primary and Secondary Sources of Copper**

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Although mined for centuries, copper (Cu) has now been deemed one of many critical minerals due to its important application in transitioning to greener energy sources. With this heightened demand for Cu and the objective to contribute to a circular economy, Cu production is rapidly growing and operations now process a variety of Cu materials from ore (primary sources) to recycled products (secondary). During production, operations are required to monitor Occupational Exposure Limit Values (OELVs) from dust emissions to ensure the health and safety of workers and limit contamination to the environment. The European Commission's Scientific Committee on Occupational Exposure Limits (SCOEL) has recently proposed to reduce the OELV several fold more stringent than existing limits; however, a recent longitudinal health surveillance study of workers at Cu smelters suggest no adverse health effects on lung function with exposure at operating conditions over the last 20 to 30 years. Given the variety of materials that are processed across Europe, exposure to workers will differ between operations. In an effort to understand the relevance of the proposed limit on a multi-operational scale, a characterization study was performed on dust exposed to workers at multiple facilities (smelters and refineries) that process different types of Cu materials (Cu ore and secondary sources of Cu powders containing Cu oxides and hydroxides). Respirable and inhalable dust was sampled from workers performing key dust generating activities around the operations. The dust was characterized using a combination of automated mineralogical analysis and chemical assay. An apportionment of Cu by species was determined for respirable and inhalable fractions. The results show significant differences in the composition of dust generated from feed materials entering the operations, but similar characteristics in composition and particle size around furnace and refining activities. At each operation, only minimal total Cu is present in the respirable dust, and where present in respirable fraction, Cu oxides are the dominant species. These findings show that interpretation of toxicology and health surveillance studies that focus on exposure to Cu oxides are relevant for setting occupational health limits, and will be conservative given that the oxides are considered the most toxic Cu species in respiratory health.

### **3.11.P-Mo182 Method Development in Elemental Analysis of Graphite from Lithium-ion Battery Recycling Process**

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Recycling is a crucial component of sustainable development and an essential step towards making batteries greener, ethical, and more sustainable and the extraction of raw materials, such as lithium and cobalt has a significant environmental and human impact. Recycling batteries can be achieved by conserving resources, reducing pollution, and contributing to the economy. Battery recycling processes involve the mechanical conversion of cathode and anode raw materials into a single powder known as "black mass" containing high concentrations of graphite processed from the battery anode composed of a copper (Cu) foil coated in graphite.

Our research with clients in the field helps monitor the efficiency of the battery recycling process through elemental analysis. Analysis of trace elements is often challenging to measure in graphite samples and thus are hard to quantify. Such methods traditionally require either the use of pressurised microwave reactors or the use of strong acids such as HF which need specialist sample introduction systems for ICP equipment in addition to strict health and safety precautions. Our research presents a workflow for trace element analyses of complex sample solutions to improve the speed and cost efficiency of the process by improving sample preparation procedures and analysis.

For trace element analysis of graphite-based materials, Inductively Coupled Plasma Optical emission spectroscopy (ICP-OES) requires complete sample digestion to clear solutions free of particulates. We aimed to characterize the elements present in low and trace amounts to improve environmental impact assessments and overcome the analytical challenges. To optimise analysis, we improve the digestion workflow and optimised a 2-step sample preparation process. The First step utilised a triple sulphuric, nitric and hydrochloric acid solution used to partially digest graphite particulates at high temperatures using a hot block. The additional process then aided preparation utilising microwave-assisted acid digestion with additional nitric acid. Samples were diluted and analysed using ICP-OES Agilent 5800 focusing on Fe, Mn, P and environmentally concerning elements like Cu, Co, Cd, Ni, Pb, Al and As. Concentration variations within different lithium-ion batteries generated sampling data used to improve plasma stability and reduce matrix effects within relevant samples. Elemental composition results were used to improve efficiency in the battery recycling field.

### 3.12.P Ionizable Organic Chemicals - Improving Risk Assessment Accuracy

#### 3.12.P-Tu246 Refining Fluorochemical Protein-Water and Membrane-Water Partition Coefficients from *in vitro* Experiments with Albumin and Phospholipids

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This study aimed to determine the partition coefficients of structurally diverse per- and polyfluoroalkyl substances (PFAS) with biological membranes and proteins using solid-supported phospholipid membranes (SSLM) and human serum albumin (HSA), as a means to enhance our understanding of PFAS distribution in organisms. The results revealed that as the fluorinated carbon chain length increased from C4 to C10, perfluoroalkyl carboxylates (PFCAs) exhibited a 0.42-log unit increase in the phospholipid membrane-water partition coefficient ( $K_{MW}$ ), while perfluoroalkyl sulfonates (PFASs) showed a 0.47-unit increase, indicating higher membrane affinity for the sulfonates. However, at longer chain lengths, no further improvement was observed, and the values stabilized to a log  $K_{MW}$  between 3 – 5. The highest HSA affinity was observed between C6 and C10, while both short-chain and long-chain PFAS exhibited decreased affinity. A comparison between experimental results and HSA – PFAS molecular docking predictions with AutoDock Vina indicated that the model effectively predicted the affinity of PFAS for HSA, especially for PFAS with lower and intermediate carbon chain lengths. Additionally, this study established a means to compare distribution coefficients between PFAS in phospholipid membranes and HSA at various starting concentrations in the water phase. The results demonstrated that at lower concentrations, PFAS were more likely to bind to HSA, while with increasing concentration, their binding tendency shifted towards the phospholipid membrane.

#### 3.12.P-Tu247 Coarse-Grained Simulations of Passive Partitioning of Ionic Surfactants into Cell Membranes

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The bioaccumulation and ecotoxicity of chemicals are key metrics in assessing environmental hazard and safety. However, for chemicals which are difficult to test such as surfactants these can be challenging to determine. Potential alternative approaches are available which use *in silico* and *in vitro* values combined with a biologically relevant partitioning parameter such as the membrane-water partitioning coefficient (log  $K_{MW}$ ). Several experimental methods exist for the determination of  $K_{MW}$  for surfactants but there are also promising computational methods available. Computational simulations, for example, utilizing free energy calculations offer potential insights, and when coupled with coarse-grained force fields such as Martini, offer the opportunity for high-throughput predictions capable of triaging a large group of molecules for risk quickly. The work described here builds on our group's automatic parameterization program for the Martini force field, *cg\_kmw*, which is then used to calculate log  $K_{MW}$ , starting from just the SMILES code of the desired solute. The structures the original *cg\_kmw* generates gave accurate predictions for a range of neutral molecules but struggled with charged species. Recently, the group has expanded this model to the most recent force field release, Martini 3, and tested it for a series of small, charged molecules. The current work outlines methodological developments and validations being performed to implement the full features of Martini 3 in *cg\_kmw*, with the goal of accessing a wider variety of ionized species. Here we present results for a cross section of 12 neutral, anionic, cationic and zwitterionic surfactants, outlining current successes and challenges in comparison to the other computational approaches for log  $K_{MW}$  prediction (e.g. COSMOmic). Results indicate that anionic and zwitterionic species perform competitively. Ongoing work is addressing some of the challenges which are identified for ionized cationic species and species with higher numbers of ethoxy groups.

#### 3.12.P-Tu249 Differentiating between baseline and excess toxicity for permanently charged compounds (ionic liquids)

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Ionic liquids (ILs) are composed of at least two permanently charged entities of which one or both are usually bulky and organic. Toxicity assessment of ILs so far focused on quantitative prediction of toxicity. Nevertheless, there is an interest in

obtaining mechanistic insights into the ILs' toxicity including the understanding of potential modes of toxic action (MOA). We predicted nominal concentration eliciting cytotoxicity in several cell lines with the aim of identifying ILs causing baseline and excess toxicity. To do so, critical membrane concentrations characteristic for baseline toxicants and a mass balance model (assuming lipids and proteins are the mainly responsible for partitioning into cells) were applied.

The general cytotoxicity of many ILs was observed at concentrations up to three orders of magnitude lower than expected by the bioassay-specific baseline toxicity QSARs developed for neutral and ionic/ionizable compounds. The possibility of metabolic activation to more toxic products was investigated but shown to be unlikely cause of higher cytotoxicity. Multiple ILs were tested within ToxCast program yet screening of the database as well as further experimental investigation of potential modes of action indicated that most of the ILs showed only non-specific activation of toxicity pathways.

It seems therefore, that especially ILs containing simple anions, either cause excess toxicity at lower critical membrane concentrations or the mass balance model use to predict cytotoxicity requires adjustment. Besides partitioning into membrane lipids via van der Waals interaction, other features of the cell membrane, e.g. surface charge or lipid composition might play a role in defining the affinity of ILs to cells. It is possible that the  $K_{mw}$  derived using POPC (1-palmitoyl-2-oleoylphosphatidylcholine) as surrogate for membranes is not suitable to describe such interactions.

Alternatively, lower concentrations of ILs, compared to other baseline toxicants, could be required to elicit the same effect. In that case the ILs should still be regarded non-specific toxicants (i.e. targeting membranes and membrane proteins as a whole and not a specific receptors).

### **3.12.P-Tu250 Intrinsic Hepatic Clearances of Selected Pharmaceuticals by Rainbow Trout S9 Fractions and Their Use in Estimating Bioconcentration Factors**

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Pharmaceutical residues are ubiquitous, often ionizable, and relatively lipophilic aquatic contaminants, many of which have the potential to bioaccumulate in fish. Besides in vivo testing (e.g., OECD305), there are several in silico methods for prediction of bioconcentration factors (BCF) in fish. However, these predictions rely on lipophilicity ( $\log K_{ow}$ ,  $\log D_{ow}$ ), and often cannot account for the impact of hepatic clearance on the predicted BCF. The OECD test guidance 319B, for determination of in vitro intrinsic clearance ( $CL_{int}$ ) using rainbow trout (*Oncorhynchus mykiss*) liver S9 subcellular fractions (RT-S9), lays the basis for inclusion of hepatic clearance in BCF extrapolation. Additionally, the S9 assays enable the study of the clearance routes in fish, whether dominated by phase I oxidoreductive enzyme systems, such as cytochrome P450, or phase II transferases.

In this study, the in vitro hepatic clearances ( $CL_{int}$ ) of fifteen pharmaceuticals (ataluren, atomoxetine, atorvastatin, azelastine, clozapine, desloratadine, felbinac, flecainide, mycophenolic acid, olaparib, orphenadrine, propranolol, quetiapine, sulpiride, zolmitriptan) were measured individually at 1  $\mu$ M concentration, using commercial RT-S9 fractions. All pharmaceuticals were tested in duplicate with active and inactivated RT-S9, firstly by including all cellular co-substrates, and secondly by excluding NADPH (the P450 cofactor) to inactivate the phase I oxidoreductive P450 enzyme system and evaluate its impact on the overall clearance rate. Of the test substances, five were not significantly metabolized in RT-S9 in the test conditions used, including clozapine, desloratadine, flecainide, olaparib, and sulpiride. Of the remaining ten substances, the metabolism of atorvastatin, azelastine, and mycophenolic acid in RT-S9 was predominantly catalyzed by phase II transferases, as their in vitro intrinsic clearances were similar with and without NADPH (P450 metabolism), whereas the other seven substances were shown to metabolize primarily via P450 system. Overall, the metabolism pathways in RT-S9 correlated well with human metabolism, although some differences were also observed. The results also demonstrate that accounting for the impact of ionization on the lipophilicity ( $\log K_{ow}$  vs.  $\log D_{ow}$ ) and the measured in vitro  $CL_{int}$ , as described in OECD319B, has a significant impact on the predicted BCF in fish, for lipophilic and metabolically cleared pharmaceuticals.

### **3.12.P-Tu251 Sorption Behaviour of (Ionizable) Organic Micropollutants to Different Sandy (Agricultural) Soils**

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In periods with limited precipitation the indirect reuse of treated municipal effluent via surface water is inescapable in the Netherlands. This is especially the case in the higher sandy soil region of the Netherlands and the agricultural areas therein. However, treated municipal effluent contains organic micropollutants (OMPs) and their fate in the environment is relatively unknown, especially for ionizable organic micropollutants. This study focussed on understanding the chemical and physical interaction between (ionizable) organic micropollutants and sandy soil, to predict the potential risk of these OMPs to groundwater resources.

Around 50 OMPs were selected based on monitoring data from Dutch municipal treated wastewater effluents. The interaction of these highly polar and/or ionizable organic micropollutants with sandy soils was researched in batch bottle experiments based on the OECD 106 guideline using five different concentrations of OMPs starting at 0.5  $\mu$ g/L and increasing with a log 3-

factor. These experiments were complemented by batch bottle experiments using a single concentration of OMPs but changing the background electrolytes. To understand the different interactions of OMPs with the different soil constituents, six different sandy reference soils were selected, containing different organic matter, Fe- and Al-oxides, and clay content. OMPs were grouped based on similar sorption behavior for subsequent data analysis. These insights and OMP sorption data on sandy soils will be used as a key input to extend an existing modeling framework developed for metal(loid)s (ORCHESTRA). We aim to extend the model's scope towards predicting the mobility of OMPs in sandy soils. In further research, the prediction will be made to fresh sandy soils and later compared to batch experiments with fresh Dutch sandy soils to see where there are still limitations in the prediction model. The development of these predictive approaches will enable an improved risk assessment for wastewater effluent-derived OMPs in research, regulation and groundwater recharge practice.

Keywords: sorption, ionizable organic micropollutants, water reuse, effluent, sandy soils, agriculture

### 3.12.P-Tu254 Bioconcentration assessment of three cationic surfactants in permanent fish cell lines

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Cationic surfactants are used in many industrial processes and in consumer products with concurrent release into the aquatic environment, where they may accumulate in aquatic organisms to regulatory relevant thresholds. Here, we aimed to better understand the bioconcentration behavior of three selected cationic surfactants, namely *N,N*-dimethyldecylamine (T10), *N*-methyl dodecylamine (S12) and *N,N,N*-trimethyltetradecylammonium cation (Q14), in the cells of a fish liver (RTL-W1) and gill (RTgill-W1) cell line. We conducted full mass balances for bioconcentration tests with the cell cultures, in which the medium, the cell surface, the cells themselves and the plastic compartment were sampled and quantified for each surfactant by HPLC MS/MS. Accumulation of the non-permanently charged amines in/to cells correlated with the surfactants' alkyl chain lengths and their membrane lipid-water partitioning coefficient,  $D_{MLW}$ . Cell-derived bioconcentration factors (BCF) of T10 and S12 were within factor 3.5 to *in vivo* BCF obtained from literature, while the cell-derived BCF for Q14 were >100 times higher than the *in vivo* BCF. From our experiments, rainbow trout cell lines appear as suitable conservative *in vitro* screening method for bioconcentration assessment of cationic surfactants and are promising for further testing of cationic surfactants.

### 3.12.P-Tu255 Deriving Fluorochemical Membrane-Water and Protein-Water Partition Coefficients from *In Vitro* Experiments with Phospholipids and Albumin

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This study aimed to determine the partition coefficients of structurally diverse per- and polyfluoroalkyl substances (PFAS) with membranes and proteins using solid-supported phospholipid membranes (SSLM) and human serum albumin (HSA), as a means to enhance our understanding of PFAS distribution in organisms. The results revealed that as the fluorinated carbon chain length increased from C4 to C10, perfluoroalkyl carboxylates (PFCAs) exhibited a 0.42-log unit increase in the phospholipid membrane-water partition coefficient ( $K_{MW}$ ), while perfluoroalkyl sulfonates (PFSAs) showed a 0.47-unit increase, indicating higher membrane affinity for the sulfonates. However, at longer chain lengths, no further improvement was observed, and the values stabilized to a log  $K_{MW}$  between 3 – 5. The highest HSA affinity was observed between C6 and C10, while both short-chain and long-chain PFAS exhibited decreased affinity. A comparison between experimental results and HSA – PFAS molecular docking predictions with AutoDock Vina indicated that the model effectively predicted the affinity of PFAS for HSA, especially for PFAS with lower and intermediate carbon chain lengths. Additionally, this study established a means to compare distribution coefficients between PFAS in phospholipid membranes and HSA at various starting concentrations in the water phase. The results demonstrated that at lower concentrations, PFAS were more likely to bind to HSA, while with increasing concentration, their binding tendency shifted towards the phospholipid membrane.

### 3.12.P-Tu256 Biomimetic Chromatography and Associated Models to Predict Biological Partitioning

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Biomimetic chromatography has the benefit of directly simulating a biological environment using commercially available high-performance liquid chromatography (HPLC) columns containing Immobilized artificial membrane (IAM) and Human serum albumin (HSA) which act as bio-relevant stationary phases. A rapid-gradient approach allows for the determination of partition coefficients for a wide variety of compounds in a single, gradient-based HPLC run. Each analytical stationary phase must first be calibrated with test mixes comprised of nonfluorinated components, each with a known Chromatographic Hydrophobicity Index (CHI) value. The CHI values for PFAS from C18 column reveals their distribution coefficients (CHI logD) as a function of pH while CHI values on HSA and IAM columns are used to determine human serum albumin binding (log  $K_{HSA}$ ) and phospholipid binding (log  $K_{IAM}$ ) coefficients. The CHI values, log  $K_{HSA}$ , and log  $K_{IAM}$  coefficients will also be determined for suspect PFAS in environmental extracts that do not have analytical standards.

### 3.12.P-Tu257 New Insights in Defining Analogy to Advance the Science of a Read-Across Framework: Starting with *Dreissena* spp. Exposed to Psychotropic Drugs

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Compounds designed to be biologically active at low doses, such as pharmaceutical compounds, raise environmental concern. Despite the amount of literature already published about their (eco-)toxicity, knowledge gaps still remain about their fate in organisms and their mechanisms and modes of action at these low environmental concentrations. Read-across is a technique for filling knowledge gaps in toxicology information by analogy between well-studied and poorly studied chemicals. This technique assumes that substances which are structurally similar will have reasonably similar physical-chemical properties, behave similarly, and elicit similar biological effects across species if their molecular targets are present. However, predictions can be difficult for aquatic invertebrates, as our knowledge on the existence and the functional roles of drug targets in these organisms is often incomplete. While direct read-across between mammals and aquatic invertebrates may be complicated, the same difficulty may exist between species phylogenetically close, since life history strategies and their ecology may influence species tolerance. The objective of our work is to address this issue on two freshwater bivalves commonly used in ecotoxicology, *Dreissena polymorpha* and *Dreissena rostriformis bugensis*. To test this, we expose organisms to different psychoactive compounds at environmentally-relevant concentrations, during 15 and 28 days; and we studied a targeted set of biomarkers, linked to important physiological functions (e.g. neuroendocrine system, energy metabolism and osmoregulation), and bivalve's ecological role (e.g. filtration and mineral contents). These functions are expected to be disrupted in bivalves according to what we already know for humans or other non-target organisms. Potential differences between males and females are also assessed. In overall, our results showed strong differences in the fate and effects of these compounds to the two species, as well as differential sensitivity between males and females. By testing the impact of several confounding factors on our capacity to read-across between psychoactive compounds and between molluscs, we bring new knowledge to improve the environmental risk assessment of pharmaceuticals.

### **3.12.P-Tu258 Applicability of a Ciprofloxacin Bioavailability Model to Four Antimicrobials: Investigating the Effects of pH and Dissolved Organic Carbon on Ecotoxicity**

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Ciprofloxacin (CIP) belongs to the fluoroquinolone family and is among the most prescribed antibiotics in human and veterinary medicine. Recent studies have shown that the ecotoxicity of CIP is affected by water chemistry factors such as environmental pH and dissolved organic carbon (DOC). In addition, a bioavailability model has been constructed to predict the impacts of the two factors on CIP ecotoxicity by incorporating the chemical speciation of CIP and the binding between DOC and the compound. Specifically, the strength of the CIP-DOC binding is predicted by the light absorption coefficient of natural water at 350 nm ( $\epsilon_{350}$ ).

This current study aims to acquire more information to expand the above knowledge to other antimicrobials. It uses cyanobacteria growth inhibition test to examine the impacts of pH and DOC on four compounds: fluoroquinolone antibiotics enrofloxacin, nadifloxacin, and moxifloxacin, which share structural similarities with CIP, and antibacterial and antifungal triclosan, which has a distinct chemical structure compared to CIP. The ecotoxicity of the four compounds will be tested on a range of pH conditions with various DOC compounds including (1) the commercial product Suwannee River Organic matter, and (2) unprocessed freshwater DOC with different concentration and compositions. Moreover, this study also investigates if the constructs of the CIP bioavailability model can be applied by other antimicrobials.

### **3.12.P-Tu259 The impact of pH on the toxicity of ionizable agrochemicals**

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A wide range of chemicals causing environmental concerns belong to the group of ionizable organic chemicals (IOCs). Among agrochemicals up to one-third are ionizable, particularly herbicides (mostly acids) and fungicides (mostly bases). IOCs can exist in the aquatic environment in two or more forms, changing their form based on pH variations determined by their acid dissociation constants (pKa). The neutral form of IOCs has a higher potential to reach organism target sites and consequently IOCs are often observed to be more toxic in this form. This indicates that part of a ionizable compounds toxicity is pH driven. Yet, while actual European surface water pH may range between 6-9, the European aquatic risk assessment does not require the pH to be fixed for aquatic toxicity tests, and only considers endpoints at a single pH, which may deviate from a worst case pH encountered in the environment. The OECD *Daphnia* guideline 202, a standard test in Tier-1 regulatory testing, usually maintains a default medium pH of 7.5 or does not account for pH variations as long as it remains within pH 6-9.

To address concerns about ionizable agrochemicals and impact of pH on tests with aquatic organisms, we first examined the 48h response of *Daphnia magna* to different buffers in the range of pH 6-9, which indicated >10% adverse effects at the more extreme pH levels of 6 and 9. Secondly, we examined the intrinsic sensitivity of *Daphnia magna* by applying the reference toxicant potassium dichromate across different buffered pH levels (6.5, 7.5, and 8.5). The LC<sub>50</sub> values for dichromate at these buffers varied 0.27-0.34 mg/L. We then investigated the toxicity of two such IOC chemicals — insecticide teflubenzuron (pKa 9.2) and herbicide 2,4-D (pKa 2.73) across the three pH levels.

The toxicity of teflubenzuron showed no significant difference among pH levels (LC<sub>50</sub> 0.30-0.67 µg/L), as expected because the neutral fraction remains dominant at all tested pH levels. However, it was noted that the chemical dissipated more rapidly at pH 7.5 and 8.5 compared to pH 6.5. The results for 2,4-D were inconclusive; at pH 7.5 and 8.5, the LC<sub>50</sub> remained comparable at 540 and 370 mg/L, resp., while no effects were observed at pH 6.5 at the highest concentration (100 mg/L), whereas a factor 10 lower LC<sub>50</sub> was anticipated at this lower pH. Our study underscores the imperative for further investigation into data requirements on intrinsic toxicity of ionizable agrochemicals at different pH levels.

### 3.12.PC Ionizable Organic Chemicals - Improving Risk Assessment Accuracy

### 3.13 Latest Science on PMT/vPvM Substances and on Bioavailability in Times of the Pollution Crisis

#### 3.13.T-01 Regulation of Persistent and Mobile substances at the European level: Regulatory developments and CLP Guidance development

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Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP), was introduced in 2008 in order to ensure a high level of protection of human health and the environment. Industrial, pesticidal and biocidal active substances fall under the scope of CLP that harmonises the classification and labelling of substances and mixtures based on their intrinsic properties by designating them into hazard categories (classes). During the last decade, substances containing the combination of persistent and mobile properties have attracted scientific and regulatory attention, facilitating entry into the water cycle and spreading over distances from local/point sources. Due to the concerns posed by such Persistent, Mobile, Toxic (PMT) and very Persistent, very Mobile (vPvM) substances, the European Commission introduced new hazard classes and labelling requirements into CLP for those substances and mixtures that came into force in April 2023, as well as corresponding scientific criteria to identify them.

The presentation will discuss the regulatory developments and detailed scientific criteria for Persistent and Mobile substances in the context of CLP and detail the development of the associated Guidance on the availability, use and weighing of all relevant information to conclude on each of the P, M and T properties. A specific focus will be placed on Mobility, as this property has just been introduced in such a wide regulatory context, with further elaborations on the relevant test methods and other available information being presented. Importantly, the comparison with the regulatory criteria for PMTs/vPvMs will be detailed, reflecting the comprehensive analysis presented in the respective Guidance, which is envisaged to be published in mid 2024. The SETAC Seville meeting will be the first time that the CLP Guidance on PMTs/vPvMs will be presented to such a wide stakeholder audience. This important communication is envisaged to raise awareness on these regulatory developments and enhance the quality of the information submitted to ECHA by Dossier submitters on such substances and, ultimately, facilitate efficient and cost-effective regulation at the European level.

#### 3.13.T-02 Occurrence of persistent and mobile chemicals in tap water

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Persistent and mobile (PM) substances are able to spread quickly in the water cycle and where thus identified as potentially problematic for the environment and water quality. If also toxic (PMT), or very persistent and very mobile (vPvM) their regulation under REACH as substances of very high concern is foreseen. PM chemicals are a challenge for a circular economy for water as they are difficult to remove from the water cycle. Yet, data on their occurrence in tap water is scarce and consequently the human exposure through drinking water consumption can only be speculated on.

In this study we analysed 33 tap water samples from 12 countries for 67 known or suspected PM chemicals. Of these chemicals 29 were detected in at least one sample with median concentrations ranging from 240 ng/L (Tetrafluoroborate) to 0.3 ng/L (2-[2-(Dimethylamino)ethoxy]ethanol). Among the detected PM chemicals 15 exceeded 100 ng/L in at least one sample with Melamine and Cumenesulfonic acid having the largest number of detects above 100 ng/L with 9 samples each.

This study shows that PM chemicals do, indeed, occur widely in tap water, in many countries, with different sources of raw water and after different drinking water treatment processes. This highlights the ability of PM chemicals to spread throughout the water cycle and consequently points towards drinking water consumption as a major human exposure pathway.

#### 3.13.T-03 Exploring Organic-Carbon-Water Partition Ratio (KOC) Data for Mobility Hazard and Exposure Assessments Using Big Data Approaches

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Chemical hazard and risk assessments often use physical-chemical properties to categorize and identify chemicals of concern. In Europe, recent legislation regarding the classification, labelling and packaging of consumer products includes new chemical hazard categories, including persistent, mobile, and toxic (PMT) and very persistent and very mobile (vPvM). While

persistence and toxicity are currently used in chemical regulation, mobility is a new criterion for chemical hazard classification. As a result, the mobility of thousands of chemicals on the global market must be assessed. Mobility is determined based on the organic carbon–water partition ratio ( $K_{OC}$ ) or octanol–water partition ratio ( $K_{OW}$ ) for neutral compounds and the octanol–water distribution ratio ( $D_{OW}$ ) for ionizable compounds. However, measured  $K_{OC}$  values can vary by orders of magnitude depending on the environmental conditions particularly for ionizable compounds.

Ideally such property data is available in machine-readable formats in standardized units with meta data regarding the environmental conditions of the measurement. However, current regulatory databases and experimental datasets have thousands of  $K_{OC}$  data values of diverging quality available, and the environmental conditions associated with this data are not easily accessible. Data must be cross-referenced to ensure the name and CAS number associated with a  $K_{OC}$  value matches the SMILES used in prediction models. The data must be tidied up to remove duplicated entries and incorrectly reported units.

In this work, we use all available  $K_{OC}$  data for chemicals on the global market in a consensus-based approach, which considers the number of  $K_{OC}$  values and their individual variability and uncertainty, and source of  $K_{OC}$  data. Data sources include the OECD's eChemPortal and QSAR Toolbox, experimental  $\log_{10} K_{OC}$  datasets, and multiple  $K_{OC}$  prediction models of varying quality and domains of applicability.

With Bayesian statistical inference approaches we aggregate values and their errors from these different sources to derive a probability distribution for the "true"  $\log_{10} K_{OC}$  value. The use of probability distributions is advantageous in these instances because we are less concerned about the environmental conditions for which the  $K_{OC}$  is measured and can apply mobility classifications based on relative risk tolerance of the uncertainty for a given  $K_{OC}$  value.

### **3.13.T-04 Lowering bioavailability with carbonaceous materials: from science to large-scale applications**

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In the 90s the discovery was made that organic compounds in soils and sediments could be bound so strongly to the solid matrix that they were hardly desorbed, and showed low available for uptake in plants and organisms, and slow transport into the groundwater. At the end of the decade it turned out that this strong binding was due to the presence of carbonaceous "black carbon" geosorbents, in particular soot and charcoal. Another important consequence in the form of limited biodegradation was revealed.

Gradually the concepts of bioavailability and bioaccessibility entered the policy domain, and technologies such as passive sampling and infinite-sink desorption, as well as bioavailability-inclusive models, were developed to include these in risk assessment procedures.

From an engineering perspective, carbonaceous materials were engineered and designed and amended to soils and sediments to reduce bioavailability and thus environmental risk. The most recent innovation is the use of biochar from organic waste materials such as sewage sludge for PFAS, PAH, PCB and dioxin remediation. Waste-based biochars have the additional advantages that they contribute to the circular economy and sequester up to half of the carbon present in the original feedstock.

Having been involved in key developments at all those stages, in the presentation I will go through the whole journey from the initial discovery of limited bioavailability, to the discovery of environmental "black carbon" being responsible for it, to the development of technologies such as passive sampling and Tenax extractions, to large-scale applications in joint-industry projects. Notable projects that will be presented include the pilot remediation of Europe's largest dioxin contamination in the Grenlandsfjords, where thin layers of carbonaceous materials reduced dioxin bioavailability by up to 90%, as well as the recent discovery that PFAS-contaminated sludge can be turned into clean biochars, that can be used for PFAS remediation of contaminated soils, while sequestering carbon. Reductions in PFAS leaching of > 99% have been found for these sludge-based biochars, and this could open up a whole new industry around the circular handling of current negative-value waste fractions.

### **3.13.T-05 Translating bioavailability science into practice through technology development and regulatory innovation in the USA**

*Upal Ghosh, University of Maryland, Baltimore County*

Pollutant bioavailability in sediment impacts uptake in the aquatic food web. While the science behind pollutant bioavailability was maturing within the scientific community two decades ago, there was little impact in the practice of sediment risk assessment that was based on total concentrations. Sediment remediation practice largely relied on disruptive sediment removal that often failed to achieve risk reduction goals. We learned from careful observations of nature that black carbon particles natively present in sediments sequestered hydrophobic pollutants rendering them less bioavailable. This observation provided powerful insights that needed to be translated into practice for both risk assessment and for designing remedies.

Translation of bioavailability science into practice had to progress on three fronts: 1) testing and development of methodologies to alter pollutant bioavailability in sediments through amendment of strong sorbents, 2) development of reliable metrics of measurement of bioavailability changes in sediments, and 3) regulatory innovation to enable acceptance of the new metric of assessing risk posed by pollutants in sediments and consideration of new approaches for in-situ management of



polluted sediments through bioavailability manipulation. This presentation will provide an overview of the developments in the science of pollutant bioavailability in the last 2 decades with a focus on the following aspects: 1) development of the novel sediment remediation technology of activated carbon amendments into sediments to reduce bioavailability that has now become a mainstream approach in the US. 2) development and standardization of passive sampling technology to measure freely dissolved concentrations as a key metric for pollutant bioavailability, and 3) regulatory innovation through active collaboration and dialog among researchers, regulators, and practitioners that enabled creation of a conducive environment where new approaches based on the latest science could flourish.

### 3.13.P Latest Science on PMT/vPvM Substances and on Bioavailability in Times of the Pollution Crisis

#### 3.13.P-Tu260 An investigation into the infiltration of Persistent, Mobile and Toxic as well as very Persistent very Mobile chemicals in Irish water

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Fear regarding water quality is a growing issue globally. As the negative effects of pollution on both the environment and health are beginning to become apparent, an emphasis is being placed on the control and mitigation of contaminants of emerging concern. Historically, persistent chemicals have been highlighted as major problem contaminants as they remain in the environment for long periods and occur in various forms. Persistent, mobile and toxic as well as very persistent, very mobile chemicals, collectively known as PMT/vPvM chemicals, are the latest persistent chemical group of concern as outlined by the EU's REACH legislation.

However, limited investigations have been carried out regarding how PMT chemicals behave in the Irish aquatic environment. This is additional interest given Ireland has one of Europe's most decentralised populations, with a large proportion of rural residents dependent on private wells and group water schemes as their primary water source. Human health is particularly at risk in these cases as Irish Water (the Irish water regulation body) carries out no water quality testing whatsoever on these systems, deferring to local governments. Hence, water treatment is not as stringent leading to contaminated groundwater.

The work here will mainly focus on agricultural and pharmaceutical sources of PMT/vPvM chemicals, as these sectors are known to be the most significant polluters. A prioritisation of PMT compounds has been selected from the 'REACH' registered substances list based on their potential hazard to both the environment and human health in an Irish context. The major pathways for these selected compounds into Irish waterways will be determined to develop robust monitoring programmes.

GIS modelling (utilising data collected from LC-MS studies) will be employed to better understand the effects of PMT/vPvM compounds on the aquatic landscape. Allowing the creation of water catchment area maps and for watershed analysis. This will provide insight into their mobility in the environment as they travel from their original sources. Various GIS modelling techniques will be used to enhance the gathered data, highlighting vulnerable and at risk areas.

From this research, several recommendations and conclusions will be drawn for future Irish legislation and research planning, creating the most effective PMT/vPvM solution possible.

#### 3.13.P-Tu261 vPvM in Urban Runoff: Barcelona as a Pilot Case

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Recently, persistent (P), mobile (M) and toxic compounds (PMTs) have gained relevance in environmental research due to their capability to reach freshwater bodies, posing great concern for the quality of potential resources of drinking water [1]. Additionally, following the REACH guidelines, a PMT subgroup based on the very persistency (vP) and very mobility (vM) substances (vPvM) represent PMTs with greater persistency and mobility in the environment. The vPvM substances involve a risk to the quality of water sources due to their vP characteristics, presence for more than 60 days in freshwater, and their vM property, log K<sub>oc</sub> lower than 3. However, the vast majority of these substances fall in the current analytical gap because of the difficulties of both, extracting and detecting them using conventional analytical procedures such as solid-phase extraction (SPE), where these very polar substances can break through, as well as the reversed-phase liquid chromatography (RPLC), the chromatography per excellence in the last decade, not always useful to retain these kind of substances.

In this study, urban stormwater runoff samples were collected throughout Barcelona. Regarding the enrichment technique consists of the vacuum-assisted evaporation of 10 mL of runoff sample at a bath temperature of 55 °C, 250 rpm platform orbital rotation and a vacuum gradient reaching a final pressure of 20 mbar. Samples were finally reconstituted into 0.5 mL of

a mixture of acetonitrile with water (95:5, v/v) and transferred to glass HPLC vials. Regarding the HPLC-HRMS, the separation of the analytes was achieved using a BEH Amide column (100 × 2.1 mm, 1.7 μm).

More than 100 samples were collected, enriched using a vacuum-assisted evaporation system and processed by LC-HRMS using a hydrophilic interaction liquid chromatography column (HILIC) and up to 27 vPvM were analysed in the urban runoff water of Barcelona. Up to 24 vPvM were detected and concentrations ranged from undetected to 271 μg L<sup>-1</sup> and acesulfame, ε-caprolactam, melamine and metformin were detected in 100% of the samples (n = 113). Pearson correlation test helped to discern patterns in the analytes of the study, showing interesting relations between compounds such as melamine and ε-caprolactam. Several positives were found, such as melamine, metformin or trifluoromethanesulfonic acid with concentrations up to 7.1, 98.2 and 2.7 μg L<sup>-1</sup>, respectively.

### 3.13.P-Tu262 The UBA list with prioritised PMT/vPvM substances in the REACH registration database

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The European Green Deal by the European Commission sets an ambitious zero pollution vision for 2050. The overall objective is to reduce pollution to achieve a toxic-free environment. The Chemicals Strategy for Sustainability Towards a Toxic Free Environment contributes towards the achievement of the Zero Pollution Ambition. The Classification, Labelling and Packaging (CLP) Regulation ((EC) No 1272/2008) has the purpose to ensure a high level of protection of health and the environment. On 20<sup>th</sup> April 2023 the new hazard classes PMT and vPvM entered into force.

In 2019 UBA published the results of a PMT/vPvM assessment for all substances registered under REACH as of May 2017 (UBA TEXTE 126/2019). Here we present the first update of the UBA list of PMT/vPvM substances in the REACH registration database. The underlying PMT/vPvM assessment now includes all substances registered under REACH as of September 2019 (UBA TEXTE 19/2023). Only 1.9 % of the unique chemical structures in the REACH registration database fall in the new CLP hazard classes PMT and vPvM.

There are 259 unique chemical structures (belonging to 360 registered substances) that were assessed as meeting the new PMT and vPvM hazard classes under CLP. The UBA list of PMT/vPvM substances in the REACH registration database is split into three priority categories.

The UBA list of PMT/vPvM substances in the REACH registration database supports registrants and downstream users to immediately act to reduce and minimise emissions throughout the whole life cycle of these substances. Local authorities at the watershed scale should use the UBA list of PMT/vPvM substances in the REACH registration database to contact manufacturers, importers and downstream users of the substances so that they are able to develop "source control" schemes. For Member State Competent Authorities, the European Chemicals Agency (ECHA) and the European Commission (EC), the UBA list of PMT/vPvM substances in the REACH registration database provides candidates for the identification as SVHC under REACH and for classifications using the new hazard classes PMT and vPvM under CLP.

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### 3.13.P-Tu263 Should we assess the P&M chemicals from a perspective of the “hazard” or “exposure”?

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Persistent and mobile (P&M) chemicals are considered emerging threats to the environment and drinking water because they can be transported over long distances, penetrate natural and artificial barriers, and resist removal by traditional water treatment procedures. Current chemical regulatory practices define P&M chemicals based on the presence of chemicals in drinking water and the potential for human exposure. However, when screening the myriad of commercial chemicals for mobility, most existing efforts utilize bright-line criteria based on chemicals' intrinsic “hazard” properties, such as biodegradation half-lives for P and organic-carbon-water partition coefficients for M. In this work, we compare and contrast the proposed intrinsic “hazard” criteria with chemicals' concentration in drinking water predicted by a fate and transport model, based on the data of 112,000+ discrete organic chemicals registered in different countries' chemical inventories. We evaluate the consistency of chemicals prioritized by “exposure” potential (chemicals with the highest concentrations in drinking water sources) and those by “hazard” assessment (chemicals with high P and M scores). We find that while chemicals with high P and M scores tend to possess a high potential to contaminate drinking water, P and M are not the only properties related to a chemical's potential to contaminate drinking water. An example is the low potential of volatile chemicals to contaminate drinking water, even if they have high scores in P and M. Thus, screening chemicals based on the P and M hazard indicators alone may, unfortunately, lead to “false positives” and “false negatives”. It is therefore not appropriate to evaluate P&M chemicals by using hazard indicators as a proxy. This work addresses the academic and regulatory need for a better understanding of the processes and properties related to drinking water contamination and for developing and evaluating

scientifically defensible methods and criteria to identify and potentially regulate chemicals of concern for the safe and sustainable use of chemicals in commerce.

### **3.13.P-Tu264 Data Science Tools for Mapping, Identification, and Assessment of PMT/vPvM Chemicals**

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Environmental authorities face an overwhelming challenge in managing and assessing risks posed by numerous manufactured chemicals. Hence, computational ecotoxicology tools like QSARs are essential in evaluating environmental hazards. Yet, for toxicity data prediction to be useful in the regulatory arena, data must be translated into synthesized information. A possible way to do this is by classifying and grouping substances. A regulatory gap exists in REACH criteria for substances with intrinsic characteristics, like PMT or vPvM substances, needing precise classification criteria to prevent potential water contamination. We have mapped over 500 vPvM/PMT suspected substances. The database included information on substances' intrinsic properties, use, tonnage, environmental release routes, process categories, and occurrence or monitoring data. We could identify substance groups that correspond well with previous classifications using standard data science techniques, such as unsupervised k-means clustering. We incorporated data quality into the mapping criteria by employing simple data analysis techniques and filtering the data regarding their origin, *i.e.*, experimental data *vs.* predictive data. Natural Language Processing models helped disentangle complex textual data regarding substance uses and identify the economic sectors in which vPvM/PMT substances occur the most. Finally, we calculated a robustness score based on the weight of evidence available for each substance serving as weights for the final classification. This work is one of the objectives of the EU Horizon 2020 project PROMISCES, which aims to protect natural resources, the environment and human health from PMTs, focusing on preventing PMT impact within the soil-sediment-water system. Mapping results will be part of a decision support framework, conveying a clear message to regulators and users regarding vPvM/PMT impacts in circular economy routes and providing targeted solutions.

### **3.13.P-Tu265 Enhancing Chemical Hazard Evaluation through Mobility Classifications**

*Colleen McLoughlin, Ziba Hosseini and Don Ward, Toxicology, Enhesa*

The Enhesa Toxicology Team manages a database of chemical hazard assessments (CHAs) with over 300000 substances that have undergone authoritative list screening and approximately 5000 substances that have verified chemical hazard profiles. To date, CHAs include 23 human health (Carcinogenicity, Mutagenicity-Genotoxicity, Developmental Toxicity, Reproductive Toxicity, Endocrine Activity, Acute Toxicity (oral, dermal and inhalation), Sensitization (Respiratory and Dermal), Irritation (Dermal, Eye, and Sensory), Systemic Toxicity, Neurotoxicity, Aspiration Potential), environmental health and fate (Persistence, Bioaccumulation, Acute Aquatic Toxicity, Chronic Aquatic Toxicity, Environmental Transformation Products), and physical endpoints (Reactivity, Flammability). Each chemical receives an overall designation called the hazard category which is derived by a weighing of endpoints and classifications. The hazard categories are represented by a stoplight system where chemicals of high concern (e.g., carcinogens) receive a red and chemicals of low concern (water) receive a green, with intermediates of yellow and yellow/green; and gray for substances of unknown concern. Our toxicology team moves in line with the Chemical Strategy for Sustainability (CSS) towards zero environmental pollution by conducting CHAs. To this end, the current works are to include mobility assessment in the chemical hazard assessments and the hazard category score. The authors identified overlapping substances between the verified substances and the work by Arp and Hale which was 1287 substances. The mobility classifications from Arp and Hale's work, (using the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) criteria and three-tiered priority levels) are being incorporated into the chemical hazard assessments. For the remaining approximately 3700 chemicals with verified hazard profiles in the database that were not analyzed in Arp and Hale's paper, we are in the process of identifying the chemical characteristics (organic vs. inorganic; types of organic structures and polarities; SMILES, etc.) to identify the chemicals and relevant information to conduct mobility assessments. The authors will present the mobility assessment conclusions, identification of PMT/vPvM substances, how the mobility assessments will be included in the hazard category, as well as the total number of chemicals that reach a red hazard category by the addition of the mobility endpoint.

### **3.13.P-Tu266 Fate and Exposure Modeling of PMT/vPvM Substances using PROTEX**

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PMT/vPvM substances have been perceived as an emerging threat to aquatic environments and human health because they have the potential to penetrate natural barriers and contaminate various drinking water sources, such as groundwater and riverbank filtrate. For this reason, it is essential to understand and characterize the environmental fate, human and ecological exposures, and potential risks for PMT/vPvM chemicals. Environmental fate and exposure models are cost-effective tools to achieve this goal, with one of the examples being PROduction-To-EXposure (PROTEX), a generic comprehensive fate and exposure model that supports modeling the fate and transport of organic contaminants in a regional multimedia environment and resulting human multi-pathway exposures. Different from "legacy" environmental pollutants, which are largely non-ionizable and hydrophobic like organochlorines and organobromines, most PMT/vPvM substances are polar or ionizable. In this presentation, we report our efforts to upgrade and evaluate PROTEX to allow for fate and exposure modeling for PMT/vPvM substances. Specifically, the latest version of PROTEX integrates more sophisticated characterization of (i) water compartments (freshwater, shallow and deep groundwater, and estuarine water) and hydrological water balance, (ii) multimedia partitioning of ionizable chemicals, and (iii) sorption of chemicals onto multiple geosorbents (amorphous organic

matter, carbonaceous organic matter, and minerals). For illustrative purpose, we apply the updated PROTEX model to predict concentrations of perfluorooctanoic acid (PFOA) in surface water, riverbank filtrate, and deep groundwater. The result shows that PFOA concentration in surface water is responsive to cessation of emissions, whereas it takes time for PFOA to be transported to and accumulated into shallow and deep groundwater. Such a time-lagging effect highlights the irreversibility of historical contamination of PMT/vPvM chemicals. By using the updated PROTEX model, one can predict the time-dependent chemical concentration in different environmental compartments. In this case, regulatory efforts can leverage this information to understand the temporal accumulation of PMT/vPvM chemicals and the irreversibility of historical contamination of PMT/vPvM chemicals. This work provides a holistic computational method to predict the environmental fate and human and ecological exposure to PMT/vPvM chemicals.

### **3.13.P-Tu267 Application of a multimedia activity model for evaluating the fate of persistent and mobile chemicals in soils: The influence of media-specific volume fractions**

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In recent years, there has been a growing focus on screening and prioritizing chemicals used in commerce for their potential to contaminate drinking water sources. The European Union, particularly the German Environment Agency (Umweltbundesamt – UBA), has proposed screening criteria to identify chemicals that may pose hazards to drinking water. These criteria aim to pinpoint chemicals that are both persistent and mobile (PM) or very persistent and very mobile (vPvM) under the EU's REACH chemical regulation. These chemicals are prevalent in various industrial sectors, making it essential to develop effective assessment tools for their environmental fate and transport. This study summarizes the development of a multimedia environmental fate and transport model, which supports simulations in a variety of scenarios, including under steady-state, equilibrium and non-steady-state and non-equilibrium conditions. The developed model includes multilayer soil and aquifer compartments (unsaturated and saturated) which is used to enable a screening level estimate of exposure of PM chemicals in coupled groundwater and surface-water systems (which might be used for water supply via “bank filtration”). Model results demonstrate the relative importance of including a saturated compartment when estimating the concentration of PM chemicals in groundwater that might be used for drinking water. The comprehensive approach developed here, thus, aims to enhance confidence in the model's performance and its utility in screening and estimating exposure to PM chemicals, which will ultimately encompass various exposure routes, including drinking water and food.

### **3.13.P-Tu268 What next for Persistent, Mobile and Toxic (PMT) substances – The plant protection products regulation as a case study for ensuring safe use concerning drinking water sources**

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PMT and vPvM have been established as new hazard classifications for CLP to indicate chemicals with potential to reach drinking water sources bypassing natural barriers like soil or riverbank sediments.

Under the new criteria for mobility the Koc-value is used as the primary metric to decide on mobile (M) or very mobile (vM) classification. However, sorption alone can only be considered a measure of the potential for a substance to be mobile, particularly if sorption is studied only in a basic laboratory test.

Many plant protection product (PPP) active substances and/or their degradation products may be considered to have the potential to be mobile or very mobile based on their Koc alone. But long standing authorisation procedures in the European Union (EU) have established comprehensive data requirement and risk assessment frameworks to ensure that active substances are only approved where they can be shown to be safe concerning human health and the environment.

True mobility is dependent on many factors such as the intrinsic properties of the molecule, the location and type of emission as well as the local environmental conditions. Therefore consideration of all available data and applying a “weight of evidence”, which is explicitly mentioned in the regulation, is essential and needs to be adequately addressed in the corresponding guidance under development.

For context a comparison has been performed of selected PPPs showing examples where a conclusion of M or vM within the CLP assessment based on basic lab sorption data is not consistent with true mobility as observed in the field.

The presentation will demonstrate that when used according to authorised use patterns supported by comprehensive data requirements and risk assessment, substances with potential mobility might not show true mobility under appropriate environmental conditions of use. Therefore, these assessments can be considered to represent an appropriate Weight of Evidence approach in the context of the mobility assessment. However, if such processes are not included in the mobility assessment, then the regulation of PPP may act as a case study to demonstrate that, when used according to authorised regulated methods, substances identified as M or vM may be safely used.

### 3.13.P-Tu269 Adsorption as a Counterpart to Bioavailability: Investigating Hydrophobic Organic Compound (HOC)–Clay Mineral Interactions to Improve Understanding of the Environmental Fate of Halogenated Pollutants

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Hydrophobic organic compounds (HOCs), such as halogenated aromatic hydrocarbons, can be very persistent in the environment and lead to adverse effects on humans and other biota. Sorption processes are highly relevant for the assessment of their environmental fate and risk, which is highly depending on the bioavailability of contaminants. In this context, especially the interactions between HOC and organic matter are intensively studied. However, significant adsorption of HOC can also take place on mineral phases [1].

For further mechanistic elucidation of HOC interactions at water/mineral interfaces, adsorption experiments with different HOC–mineral systems were performed. Five halogenated benzenes ( $\log K_{OW}$  2.6–6.5) were used as HOC representatives and 20 smectite-rich bentonites were used as mineral phases.

Results showed a large mutual influence of the characteristics of HOC and clay minerals on the extent of sorption. The determined solid-liquid adsorption coefficients  $K_d$  varied over several orders of magnitude for specific HOC–mineral interactions. The hydrophobicity of the HOC played a relevant role for the level of adsorption, but to a lesser extent than the mineral properties. This can be illustrated by the example of two selected HOCs (A:  $\log K_{OW}$  2.6 and B:  $\log K_{OW}$  5.6), whose adsorption varies depending on the clay minerals by up to a factor of 5 for substance A ( $\log K_d$  1.8–2.5), but by up to a factor of 90 for substance B ( $\log K_d$  2.1–4.0).

The interpretation of results is based on various clay mineral characteristics. In terms of particle morphology, especially the pore size distribution is discussed as factor for the interaction of HOCs with clay minerals. The sorption processes are further interpreted on the basis of molecular simulations [1,2].

The results indicate the general influence of clay minerals on the adsorption of HOCs and suggest a great influence of specific HOC–clay mineral interactions on the environmental distribution behavior of HOCs with potential relevance for the fate and transport of HOCs in the environment, i.e., for both assessing bioavailability and long-term source/sink phenomena in soils and sediments. The elucidation of the fundamental processes is also critical for the use and modification of adsorbents with respect to potential remediation treatments.

[1] Böhm et al. (2023): <https://doi.org/10.1007/s11356-022-24818-4>

[2] Grančič et al. (2023): <https://doi.org/10.3390/min13020280>

### 3.13.P-Tu270 On-site solid-phase extraction of polycyclic aromatic compounds (PACs) from biochar-amended contaminated soil.

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Soil has the potential to act both as a sink and as a secondary source for contaminants in the environment. This is due to sorption processes (ad/absorption and desorption) and the possibility of contaminants to desorb from the soil constituents to soil porewater and be further leached out to groundwater and surface water. This leachability is, however, dependent on different parameters, e.g., physio-chemical properties of the compounds, soil type, and contamination age. Furthermore, the soil's content of organic matter has an impact on the leaching as dissolved organic carbon (DOC) may serve as contaminant carrier and thereby increase the contaminants' mobility and make them more (bio)available. Therefore, soil porewater can be a useful matrix to study the leachability of environmental contaminants like polycyclic aromatic compounds (PACs) from contaminated soil.

PACs are often present at trace levels in water samples (e.g., groundwater, surface water, soil porewater) taken at/near historical contaminated sites, requiring sampling of large volumes of water to exceed the detection limit. Besides the large volumes required, these samples should preferably be collected in glass vessels (to minimize losses of analytes due to ad/absorption) which can make the transport and storage of samples cumbersome; additionally, PACs have also been observed to adhere/sorb to glass surfaces making conventional water sampling in glass vessels problematic.

In this study, a field study was set up to investigate biochar's ability to immobilize PACs in biochar-amended contaminated soil. For sampling soil porewater as a measure of the leachability and thus the (bio)availability of PACs, an on-site solid-phase extraction (SPE) sampling technique was evaluated. The soil porewater was sampled using a PRENART stainless steel lysimeter embedded into the soil and enriched onto an Waters™ HLB SPE cartridge. Additionally, a laboratory study was conducted to validate the robustness (i.e., examining whether increased DOC and PAC concentrations as well as increased volume passed through impacted PACs' sorption on the SPE sorbent) of the on-site sampling method. The findings from this study will emphasize the advantages of utilizing an on-site SPE sampling method, e.g., simplifying transport and storage and minimizing loss of PACs as they are already enriched onto the SPE sorbent.

### 3.13.P-Tu271 Scrutinising Soil: A Cost-Effective, Scalable Approach to Investigate the Behaviour of Small Molecules in Soil Ecosystems Using Chemical Sampling Probes

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A major source of pollution in soil comes from anthropogenic organic contaminants, often via point sources, like direct application of chemicals and non-point sources such as irrigation and fertilization. Their chemical properties, environmental conditions, and soil type affect the exposure and fate of these pollutants. Providing a diagnostics tool that can characterise the chemical and biological mechanisms affecting soil health will contribute towards understanding and remediating soil efficiently.

Passive sampling devices (PSD) have been manufactured using 3D printing for use in environmental matrices like air and water but have not yet been utilised in soil. The presented work provides a proof-of-concept methodology that uses 3D-printed PSDs to characterise the transport behaviour of contaminants of emerging concern (CECs) in soil, after wastewater (WW) exposure. Finally, the effects of WW on soil microbial activity were investigated using a cotton strip assay. The PSDs were assembled with five individual 9 mm hydrophilic-lipophilic balanced (HLB) sorbent disks and a protective PES membrane, buried in the soil (LufaSpeyer 2.2, Germany) and sprayed with periodic doses of WW (10ml/3hrs). Contaminant uptake, for 17 compounds, onto the HLB sorbents was determined using a targeted direct-direct-injection rapid liquid chromatography-mass spectrometry (LC-MS/MS) method; reducing the need for pre-treatment such as complex soil extraction and clean-up analytical techniques.

From the 160 target compounds, 33 (27 quantified) were detected in the WW influent sample. Benzoylcegonine (BZE) was detected at the highest concentration at  $8,863 \pm 23$  ng/L. Seventeen CECs were quantified in the PSD extracts, the highest concentrations were found for carbamazepine(cbz)-10,11-epoxide at  $9.2 \pm 0.50$ , followed by BZE at  $5.9 \pm 0.25$  ng/disk. Soil leachate samples were also analysed, 11 CECs were detected the highest being BZE at  $346 \pm 4$  ng/L. Cbz-epoxide was detected only on disk, whereas haloperidol and nortriptyline were detected in WW and on disk but not in leachate. The cotton strip assays showed faster degradation of the strip when the soil was exposed to WW after two weeks compared to non-dosed soil. The use of 3D-PSDs for soil sampling represents a new method which can be rapidly upscaled for large monitoring programmes for chemical transport and fate studies.

### **3.13.P-Tu272 Soil amendment with Sargasso biochar: a way to secure animal production in the antillean chlordecone contamination context?**

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The French West Indies faces two major environmental and health problems: on the one hand soil and food products chlordecone pollution, a pesticide widely used from 1970 to 1993, and on the secondhand arrivals of invasive brown algae, *Sargassum* spp. One way to manage the abundance of this biomass, as well as to limit the transfer of the CLD still present in the soils, can be the addition to these soils of biochar (BC) or activated carbon (AC) made from Sargasso. This study purpose is to evaluate the reduction of CLD transfer from soil to animal following soil amendment by BC of Sargasso (BCS) or AC DARCO (ACD, a matrix known for its sequestration power). Oral bioavailability measurements were carried out on laying hens and piglets fed respectively with granules containing a fraction of contaminated soil with or without BCS or ACD amendment. After a contaminated period of 10 and 21 days for the piglets and the laying hens respectively, the animals were anaesthetized by electronarcosis and target organs, liver, fat and muscle, were collected and analyzed. For piglets, only liver data revealed significant reductions in bioavailability between modalities for both soils. For the liver the most important reductions were obtained in Andosol and were of the order of 41% with the BCS and 65% with the ACD. For Nitisol a smaller reduction, at the limit of the significance, is observed with the BCS (10%) while with the ACD one obtains a reduction around 57%. In laying hens, liver, egg yolk and muscle analyses showed similar rates of bioavailability reduction by modality. Thus, with BCS reductions of 26 to 31% were observed and with ACD the reductions were higher, in the order of 56% to 59%. The difference between the two soils can be explained by much higher CLD concentration in Nitisol than in Andosol, which can limit the effectiveness of Sargasso biochar by saturation. The variations of physico-chemical characteristics that exist between the two matrices tested, resulted from the additional activation phase for DARCO. It may explain the difference in efficiency between the two matrices tested. This experiment is considered as a first step to judge the relevance of this rehabilitation technology in the case of the French Antilles. These promising results toned to be tested in situ in order to verify the feasibility and the reduction levels obtained in real Caribbean conditions.

### **3.13.P-Tu273 The Challenges of Validating and Implementing Methods for Characterising Non Extractable Residues in Soils**

*Avril Crowe, Metabolism, Labcorp, United Kingdom*

According to IUPAC the definition of Non Extractable Residues (NER) in soils is species originating from chemicals that remain unextracted by methods which do not significantly change the chemical nature of the parent substance or its degradates or the structure of the matrix. Regulatory opinion on NER formation differs considerably. Pesticide regulations have historically considered NER to be irreversibly bound degraded residues of no environmental concern. However, ECHA

considers NERs as potentially bioavailable, non-degraded residues (parent substance) unless it has been demonstrated that the chemical is completely degraded or irreversibly bound.

There has been some discussion about the methods of extraction which should be employed prior to quantifying the amount of NER remaining in the soil. Some of these methods can be considered harsh and may not conform to the recommendation not to alter the nature of the chemical or matrix.

Where the amount of NER is 10% of the applied radioactivity or more ECHA considers that it should be further characterised by separating it into Type I, Type II and BioNER:

- Type I NER is considered to be physically entrapped or non-covalently bound and potentially bioavailable.
- Type II NER is considered to be covalently or irreversibly bound.
- BioNER (Type III) is considered to be incorporated into the soil biomass and of no concern.

Methods for differentiating Type I NER using silylation and EDTA extraction have been proposed. In order to determine the amount of Type II NER it is necessary to quantify the BioNER by concentrated acid hydrolysis followed by calculation of the difference. However, there are currently no suitable standardised methods for quantifying the amount of NER or the different types of NER for use in regulatory studies.

The poster will discuss the challenges associated with developing and validating methods that might be suitable for use in regulatory studies.

### **3.13.P-Tu274 Uptake and elimination of per- and polyfluoroalkyl substances in earthworms exposed to a fortified sandy loam soil**

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We investigated the uptake and elimination kinetics of per- and polyfluoroalkyl substances in earthworms exposed to amended artificial soil to derive bioaccumulation factors (BAF). Uptake kinetics was determined at two concentrations, 0.01 and 1 mg/kg, of each of 18 PFAS relevant to the DoD (8:2 FTS, PFBA, PFBS, PFDA, PFHpA, PFHpS, PFHxA, PFHxS, PFNA, PFOA, PFOS, PFOSA, PFPeA, PFTeDA, PFTrDA and PFUdA). Exposed earthworms were sampled in triplicate after 1, 3, 7, 14, 21 and 28 d. The concentration of PFAS remained relatively unchanged during the exposure and rates of uptake were estimated for most individual PFAS. For the 0.01 mg/kg treatment, steady state was reached (based on no significant difference between the last three endpoints) for all PFAS except PFHpS, PFHxS, PFOS, PFTeDA (most tissue samples below detection), and PFTrDA. For the 1 mg/kg treatment, steady state was reached for all PFAS except 8:2 FTS, PFDA, PFTeDA, and PFUdA. Reliable rates of uptake were obtained from all PFAS except for PFHxA and PFPeA. Uptake rates were lower for the 1 mg/kg (typically by a factor of 3 to 4) for all individual PFAS except 8:2 FTS, likely because of sublethal physiological impairment caused by the exposure to a high concentration of sum PFAS (i.e., approximately 10 mg/kg) in the exposure soil. For the 0.01 mg/kg treatment, BAFs ranged from 4 to 49 for most PFAS, but were higher for PFHxS (72), PFTeDA (271), and PFUdA (299). BAFs were lower for the 1 mg/kg treatment for all individual PFAS, typically by a factor ranging from 3 to 16. The elimination kinetics experiment will also be derived. Elimination rates were used to calculate kinetically derived BAF values as the ratio of uptake and elimination rate constants.

### **3.13.P-Tu275 Assessment of the Persistence, Bioaccumulation, Mobility and Toxicity of cosmetic ingredients in the COSMETICK database: a preliminary analysis of regulatory evolution**

*Juliette Guerineau, Clarisse Bavoux, Cyril Durou, Faizan Sahigara and Kevin Georges Antoine Bonnot, Consultancy for Environmental & Human Toxicology and Risk Assessment (CEHTRA), France*

Faced with growing environmental challenges, regulations on cosmetic ingredients are evolving. The assessment of the environmental and ecotoxicological impact of these products has become essential. The COSMETICK tool marks a significant step forward in the toxicological and ecotoxicological assessment of cosmetic products. By integrating over 800 ecotoxicological profiles, this tool aims to fill the gap in the assessment of the ecotoxicological impacts of cosmetic ingredients, often underestimated in cosmetic regulations. It assesses these substances using the criteria of PBT (Persistence, Bioaccumulation, Toxicity) and PMT (Persistence, Mobility, Toxicity), reflecting an approach similar to regulation evolution under the EU Chemicals Strategy for Sustainability, anticipating the application of PBT/PMT assessment in regulation such as EU REACH and CLP.

The first part of the study involves a descriptive statistical analysis, sorting ecotoxicological data and visualization to extract maximum information. Based on complete datasets of ecotoxicological criteria, analysis of the distribution of ingredients according to these PBT and PMT criteria, reveals the proportion of ingredients failing to pass one or all of the criteria. In the second part, we introduce an innovative hierarchical classification method on principal components (HCPC), based on chemical families. It uses the clustering of molecular fragments (qualitative data) and 2D descriptors (quantitative descriptors) and is enriched by unsupervised statistical analysis methods, such as PLS regression. This approach results in a chemical mapping of cosmetic compounds, establishing a correlation between molecular structure and ecotoxicological alert levels.

This study provides a unique perspective on the environmental impact of a broad spectrum of cosmetic compounds and ingredients of varying quality. By focusing on molecular structure, it enables the ecotoxicological impact of substances to be predicted qualitatively. From the clusters created and the descriptors and fragments characterizing these groups, we will also be able to identify ranges of molecular descriptors critical for ingredient assessment. Such information is essential for effective and proactive environmental assessment and green formulation in the cosmetics industry.

### **3.14 Lower Micron and Nanosize Plastics: Challenges, Analytical Methods, Occurrence, Composition, Local Sources, Long-Range Transport and Human Exposure**

#### **3.14.T-01 Beyond the Horizon: Unveiling Transport Mechanisms and Residence Times of Atmospheric Micro- and Nanoplastics**

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Plastic pollution, a pervasive global challenge, necessitates a nuanced understanding of the atmosphere's micro- and nanoplastics (MNP) transport dynamics to predict residence times and assess their potential for long-range atmospheric transport under different meteorological conditions. This study intricately examines MNPs transport mechanisms and residence times, accounting for diverse compositional, dimensional, and meteorological variables. From 1 nm to 5 mm, MNPs present unique complexities, particularly in tropospheric transport and deposition. MNPs demand dedicated or adapted equations and parameters, a necessity heightened by non-spherical shapes and complexities arising for aggregated MNPs.

Addressing the density, size and shape spectrum of MNPs, this study theoretically investigates and advances the description of different deposition processes: dry settling, resistance deposition near surfaces, and wet deposition during precipitation events.

For each process, the impact of MNPs size, density, and shape is assessed and compared to meteorological or other environmental influences (e.g. wind speed, rain intensity, deposition surface properties):

- Dry Settling: Exploring dry settling reveals size, density, weathering, shape, and aggregation impact MNPs' behavior.
- Dry Deposition: With a specific impact of land surface characteristics and atmospheric stratification on the MNPs deposition.
- Wet Scavenging: Scrutinizing below-cloud and in-cloud processes, these investigations unravel intricate mechanisms of particle captured.

Finally, we combine the above processes to determine the total residence times of MNPs in the atmosphere under selected meteorological scenarios. This research augments current theoretical frameworks for predicting the atmospheric transport of MNPs, providing valuable process descriptions for environmental fate models and directives for monitoring efforts. A systematic exploration of MNPs characteristics and environmental conditions enhances our scientific comprehension of atmospheric MNPs transport, supplying essential insights for refining transport models and steering future investigations.

#### **3.14.T-02 A realistic nanoplastics test material: accelerated ageing, production and phys-chem characterization**

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Research on Nanoplastics (NPs) has still many knowledge and methodological gaps, mainly due to the lack of standards and realistic test materials which negatively influence development of analytical techniques for the detection and quantification of NPs in environmental, food and human samples. Moreover, toxicological and ecotoxicological studies cannot be conducted in an appropriate way. In literature, there are still very few cases of NPs isolated, detected and quantified in environmental and human samples. Most of the work related to NPs done so far has been done using polystyrene latex beads. Unfortunately, these particles are not representative of realistic NPs mainly because secondary NPs generated from fragmentation are subjected to different stressors, modifying the original structure and composition of the polymer, as well as their surface properties, influencing their stability, protein corona formation and consequently, their fate in the environment and in the human body. Moreover, latex beads are colloidal dispersions stabilized with surface charge moieties and surfactants, while realistic NPs are fragments that could be stabilized by natural processes. Several researchers attempted to produce environmentally relevant NPs using both top-down approaches (by the degradation of larger plastic particles) and bottom-up via direct NPs synthesis. Reference particle models are essential to obtain an accurate perspective of how environmental nanoplastics (NPs) behave in natural systems and to generate data on their environmental fate and impact on living organisms. However, although the current available models are particularly important for filling the initial knowledge gaps on nanoplastics, they do not demonstrate enough diversity to represent the actual heterogeneity of the physical and chemical properties of environmental nanoplastics. In this contribution, the research activities about model environmentally relevant neoplastic materials synthesis and characterization are presented concerning identification of a novel strategy for high throughput production of model nanoplastics with properly engineered surface chemistry and morphology that could mimic naturally occurring NPs. Artificially aged plastic litter (UV exposure + Cryomilling) is discussed with specific insight into the



surface chemistry modifications engineered in order to mimic external stressors influences on nanoplastics objects as they are exposed to the environment.

### **3.14.T-03 Automated Microplastics Identification (<500nm to mm's): Particle Shape/Size Artefact-Free Submicron IR & Simultaneous Raman with Nile Red Fluorescence Imaging to Pre-Scan Samples for Increased Speed**

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Microplastic (MP) contamination have been recognized as a global environmental problem. MP particles are found globally in water, air, soil and regularly ingested by marine life. MPs can enter the human body by via contaminated water, beverages, and food, and by breathing airborne particles. The MP research community has grown quickly to address questions related to environmental/health risks. Spectroscopic analysis is frequently used to characterize populations of MPs, but most IR microspectroscopic analyses have been limited to >20 µm particles. Better spatial resolution techniques such as Raman microscopy exist, but often suffer from limited sensitivity and/or autofluorescence issues. This has left a critical unmet need in the analysis of micron scale MP particles, which are of particular concern for human and animal health, because these particles are able to pass through the gut wall to accumulate in tissue with potential impact to organ function.

We have developed an optical microscopy based platform with automated capability for the measurement and analysis of micron scale MP particles based on Optical Photothermal Infrared Spectroscopy (O-PTIR) and complementary and simultaneous Raman spectroscopy. Preparations of microplastic particles can be automatically screened via optical microscopy (or Fluorescence imaging with Nile Red) to identify particles of interest and then automatically measured by O-PTIR and/or Raman. While conventional infrared spectroscopy (and even Raman) can struggle to spectroscopically identify micron scale MP particles, the O-PTIR approach overcomes the spatial resolution limits of conventional infrared spectroscopy by using a photothermal detection mechanism that employs a separate visible probe beam to detect infrared absorption. Because of the smaller wavelength or the probe beam, O-PTIR can achieve spatial resolution 10-30X smaller than infrared diffraction limits, while also avoiding size and shape dependent scattering artifacts that often limit the repeatability of traditional FTIR/QCL based techniques. O-PTIR is also insensitive to autofluorescence which can be problematic with Raman.

This presentation will review O-PTIR technology and operating principles and then discuss the automated measurement and analysis of arrays of MP particles whilst providing key measurement performance metric

### **3.14.T-04 Differentiating Microplastics from Natural Particles in Aqueous Suspensions using Flow Cytometry with Machine Learning**

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Microplastics (MPs) in natural waters are heterogeneously mixed with other natural particles, including algal cells and suspended sediments. An easy-to-use and rapid method for directly measuring and distinguishing MPs from other naturally present colloids in environmental samples would expedite analytical workflows. Developing such an approach would be indispensable for both screening for the presence of MPs and quantitatively identifying and enumerating them, contributing to a better understanding of their presence in environmental and technical systems. This is particularly relevant for small sized MPs (e.g., < 20 µm), which are not routinely measured by analytical approaches used in the field today, such as µFTIR. In this study, we established a database of MPs scatter and fluoresces properties, either alone or in mixtures with natural particles, by stain-free flow cytometry. The resulting high-dimensional data were analyzed by machine learning approaches, either unsupervised (e.g., visual stochastic network embedding) or supervised (e.g., random forest algorithms). We assessed our approach in identifying and quantifying model MPs of diverse sizes, morphologies and polymer compositions in various suspensions including phototrophic microorganisms, suspended biofilms, and sediments. We could precisely quantify MPs in microbial phototrophs and natural sediments with high organic carbon by both machine learning models, although it was not possible to distinguish between different MPs sizes or polymer compositions. By testing the resulting method in environmental samples through spiking MPs into freshwater samples, we further highlight the applicability of this approach to be used as a rapid screening tool for MPs. Collectively, this workflow can be easily applied to a diverse set of samples to assess the presence of MPs in a time-efficient manner.

### **3.14.T-05 Analysis of microplastics in underground waters and drinking waters down to 5µm**

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“Human beings ingest 5 grams of plastic per week”. The press reported this statement even before the WWF-funded study was published. It turns out this figure corresponds to the worst-case scenario, hardly representative of reality! Many press articles highlight microplastics contamination of bottled water but also in tap water and point out a potential risk to human health. However, occurrence of microplastics in underground waters and the efficiency of drinking water treatment plants (DWP) are not well known yet. This study focuses on two French DWPs with underground waters as resources. The first plant has a 6 m deep resource influenced by a close-by river. For the second plant, two protected resources (70 m and 14 m depth), were analysed. A dedicated filtration system was used to sample large volumes of water (>500 L) on 5 µm mesh-size stainless steel filters and is presented. This system includes a filter dedicated for the field blank sample. Microplastics were quantified with state of art technique, namely imaging µFT-IR with a 3.3 µm pixel resolution at Aalborg University (Simon et al., 2018,

Kirstein et al. 2021). Results show small amounts of microplastics in both underground waters and drinking waters. Drinking waters present a contamination of microplastics >6.6 µm below 1 MP/L. Underground water influenced by the river (inlet DWP1) had a higher amount of microplastics compared with inlet waters from DWP2: 2 MP/L versus 0.3 MP/L, respectively.

### **3.14.P Lower Micron and Nanosize Plastics: Challenges, Analytical Methods, Occurrence, Composition, Local Sources, Long-Range Transport and Human Exposure**

#### **3.14.P-Mo183 Overcome the Obstacle of NP Analysis – A Concept of Artificial Intelligence Combined with Chemical/Microscopic Methods**

*Alexandra Foetisch, Collin Weber and Moritz Bigalke, Technical University Darmstadt, Germany*

Numerous studies have shown the potential risk that nanoplastic (NP) represents for the living organisms in the different ecosystems. However, the amount and characteristics of NP present in the environment are still unknown in its full extent. Even if several methods have already managed to quantify or characterize environmental NP, none, to our best knowledge, could yet provide a complete characterisation over the full nanoscale range combined with a high sample throughput.

The present work tackles the challenge of NP full characterisation in a wide range of media (air, water, soil) by testing an innovative combination and alignment of µ Raman spectroscopy (RS), scanning electron microscopy coupled with energy dispersive x-ray spectroscopy (SEM/EDX), pyrolysis gas chromatography mass spectrometry (Py-GC/MS) and artificial intelligence (AI). The aim is to use the RS and Py-GC-MS data to train an AI model to be able to automatically characterise NP in environmental samples using SEM/EDX data. Since this project is in its infancy, only the concepts are presented here.

The method is tested and optimized using sample types of increasing complexity, starting with pure NP, mixed NP, spiked media and, finally, environmental samples. First, NP chemical information is acquired down to a ~500 nm size using RS and NP quantification, size and surface characteristics are obtained using SEM/EDX down to a ~20 nm size. Based on the RS and SEM data, the total mass of each polymer present in the sample is extrapolated and then cross-validated by performing a Py-GC/MS analysis on the same sample. Monte Carlo simulations are then used to model the error of the extrapolation based on the 2 and 3D data provided by the RS and SEM data.

Finally, the dataset acquired with RS and SEM/EDX on >500 nm NP is divided into a training and testing set to build a convolutional neural network (CNN) allowing the differentiation between NP and non-NP particles present in a sample. The aim of this model is then to allow the identification and characterisation of <500 nm NP present in a sample using SEM/EDX data and AI and cross validation with Py-GC-MS data.

In case of success, this model would provide for the first time a full characterisation of environmental NP with a high sample throughput. This methods combination could then provide a more accurate assessment of the NP pollution in the environment.

#### **3.14.P-Mo184 Development and validation of a pyrolysis - gas chromatography – high resolution mass spectrometry method for the determination of nano- and microplastics in river water and sediment samples**

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Micro- and nanoplastics (MNP) are a class of emerging contaminants in the environment. Measuring concentrations and sizes of micro- and nanoplastics in the environment is essential to assess the risks plastic particles could pose. Microplastics have been detected globally in a variety of ecosystems. Their determination, however, is still challenging. Recently, pyrolysis gas chromatography to mass spectrometry (Py-GCMS) was introduced for mass related quantification of microplastics. Under high temperature and oxygen-free conditions thermal breakdown of polymers takes place and characteristic volatile pyrolytic products are generated. Under reproducible pyrolysis conditions following gas chromatographic separation and mass spectrometric detection, individual polymer types can be quantified. Pyrolysis GC-MS has shown to be promising for the detection of MNP in environmental samples and can give information on the different polymer types and quantities. In this study, different commonly occurring MNP polyethylene (PE), polypropylene (PP), polystyrene (PS), polymethylmetacrylate (PMMA), polybutylmetacrylate (PBMA), polyvinylchloride (PVC), polyethylene terephthalate (PET), polytetrafluoroethylene (PTFE) were investigated. The preparation of standards was optimized by comparing cryo-milling with quartz sand and dissolution in various solvents. A low resolution pyrolysis GC-MS method was developed and transferred to a py-GC-HRMS Orbitrap system. Both methods were validated. Furthermore, a double-shot method was developed to qualitatively determine volatiles additives present in the MNP. As an application, pre-concentrated nanoplastics from aqueous environmental samples (surface water and river sediment) were qualitatively and quantitatively investigated with the optimized methods. For all MNP except PP three indicator pyrolysis products were found and limits of detection were below 0.02 ng absolute in all cases, with R<sup>2</sup> values ranged between 0.990 and 0.999. With the optimized py-GC-HRMS method PS and PE were successfully identified in sediment samples and PET in the water samples.

### **3.14.P-Mo186 Development of Semi-automatic Analytical Methods for Fine Microplastics larger than 1 $\mu\text{m}$ in Surface Water by Raman Imaging Microscopy**

*Yutaka Kameda, Chiba Institute of Technology, Japan*

It is a matter of great concern that ultra-fine microplastics (UFMPs), ranging from 1  $\mu\text{m}$  to 20  $\mu\text{m}$ , may have adverse effects on human health and wildlife. The occurrence of UFMPs in the ocean poses issues that should be addressed in the context of ecological risk assessments in aquatic environments and environmental behaviors in marine settings. However, quantifying and identifying UFMPs in water samples is challenging because each particle must often be individually identified by operators, requiring a significant amount of time and effort. In addition, Raman spectroscopy is not suitable for observing a wide range of areas to quantify and identify all UFMPs in samples, leading to high extrapolation for estimating UFMP concentrations and their upper limits of detection. Pretreatment processes for UFMPs with minimal contamination are also necessary. This presentation introduces semi-automatic analytical methods for UFMPs in the surface water of Tokyo Bay, including pretreatment methods. The pretreatment process for the water samples consists mainly of three crucial steps. The first step involves removing organic fluorescent matrices from glass apparatus and two types of membranes, achieved by heating them at 450 degrees Celsius for 2 hours. Subsequently, surface water samples were passed through a silver membrane to collect UFMPs, which are then gathered on a nickel membrane. The second step involves a digestion process using the Fenton reaction in a centrifuge tube. Following this, sodium iodide is added to the tube for density separation. The third step aims to remove excess sodium iodide and other salts in supernatants from the tube, obtained three times after centrifugation. The pooled supernatant is filtered by a nickel membrane, and hydroperoxide and hydrochloric acid are added to dissolve excess salts. Finally, the washed solution, including FHMPs, is passed through an area with a 4 mm diameter of a silicon membrane. UFMPs on the membrane can be analyzed using the DXR3xi Raman Imaging Microscope (Thermo Fisher Scientific). The Raman microscope has ultra-fast chemical imaging capabilities, making it possible to analyze UFMPs across more than 63.7% of the total area for 32 hours. Using this analytical method, microplastics as small as 0.3  $\mu\text{m}$  could be detected. The poster will also show recovery tests conducted in our laboratory, as well as monitoring results of UFMPs in Tokyo Bay.

### **3.14.P-Mo187 Validation of Nanoplastic Extraction from Compost by Advanced Techniques and Screening Techniques to Enable Nanoplastic Identification and Quantification by Nanoscale Infrared Imaging**

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Environmental aging of macro- and microplastics might lead to formation and release of nanoplastics. However, current extraction protocols established for microplastics are not suitable for nanoplastics because of (hetero)agglomerate formation and longer flotation times during density separation. In addition, common microscopy counting techniques for microplastics (Raman, IR, Fluorescence) are not applicable to nanoplastics, and the mass-based detection limit of techniques such as thermogravimetric analysis (TGA) or pyr-GCMS corresponds to an unsatisfyingly high number concentration of nanoplastics that would remain undetected, if present. Consequently, there is a knowledge gap on nanoplastic presence and quantity in different environmental compartments.

Here we present an extraction process developed for polyamide nanoplastics from compost, which is based on deagglomeration and fractionation by size to isolate the nanoplastics from compost components, which are then enriched by a stacked density-separation. The extraction protocol was validated by different advanced and screening techniques, using spherical fluorescent polystyrene (PS) beads (0.91  $\mu\text{m}$ ) for visual inspection in a first approach, then gravimetric approaches including TGA for polyamide (PA) nanoplastics and finally analytical ultracentrifugation during density gradient separation. Each step of the extraction protocol was validated separately to identify and improve the most critical steps. For demonstrating the recognition of individual nanoplastics via infrared nanoimaging using Scattering-type Scanning Near-field Optical Microscopy (s-SNOM), we spiked polydisperse, non-spherical pristine and UV-aged PA nanoplastics into compost < 20  $\mu\text{m}$  and extracted them with stacked density separation. Imaging was performed at few specific IR frequencies, which were obtained from spectra of the reference materials (PA and compost particles). The reference spectra were recorded by Fourier transform infrared nanospectroscopy (nano-FTIR). Based on the IR images, the individual nanoparticles were identified and counted using special data and image processing.

### **3.14.P-Mo188 Capillary Electrophoresis as a Promising Technique Towards a Universal Analytical Tool for Separation and Detection of Nanoplastic Particles**

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Nanoplastics are released throughout the entire life cycle of plastic consumer products. Nanoplastic size is small enough to avoid plastic removal procedures and to penetrate biological barriers, increasing the possibility of inherent toxicity and toxin transportation to organisms. Due to reactivity with other environmental materials, nanoplastic quantification in natural items becomes more difficult.

In the framework of the EU-funded CE4Plastics project, capillary electrophoresis is the main technique for the nanometrological approach herein described, in which separation of differently sized nanoplastic particles is reported. A separation methodology for polystyrene nanoparticles with particle diameters ranging from 30 to 300 nm has been

developed, making use of a buffer containing 5 mM sodium phosphate in conjunction with 5 mM sodium dodecyl sulphate in alkaline medium (pH=8.9), through a bare fused silica capillary and ultraviolet detection. The proposed strategy was also assessed for polymethyl methacrylate, polypropylene, and polyethylene nanoparticles, finding that stronger alkaline conditions, employing an ammonium hydroxide buffer (7.5%, pH=11.9), enabled the separation of these polymers for the first time by means of electrophoresis for particle diameters below 200 nm. Detection limit was found to be in the concentration region of  $10^{11}$  particles  $\text{mL}^{-1}$ , whilst repeatability obtained for migration time and peak area had variations in all cases below 10%.

Data on nanoparticle effective electrophoretic mobility show an increasing absolute value from the smaller to the larger sizes. In contrast, the absolute value of particle surface-charge density is decreasing with the increasing particle size. This phenomenon aligns with shorter migration times revealed by the smaller particles, whilst the larger particles migrate later due to their smaller absolute value of surface-charge density and higher absolute value of effective electrophoretic mobility. It is believed that the separation mechanism involves a combination of linear and nonlinear electrophoretic effects. This work is the first report on the quantification of nonlinear electrophoretic effects on nanoplastics in an electrophoresis system and presents an interesting contribution in nanoplastic research by electrophoresis.

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### **3.14.P-Mo189 Navigating Nanoplastics: Challenges and Approaches in Producing Standardized Materials**

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Plastic is considered one of the most practical inventions of the 20th century, yet today it represents one of the greatest potential threats to the environment and human health. The low production cost of plastics, as well as their very favourable characteristics, including light weight, durability, inertness and resistance to corrosion and degradation, have led to significant technological and social progress. Although concerns regarding the environmental impact of microplastics (MPs) surfaced in the 1970s, the scientific community has recently recognized the pressing urgency of the issue, particularly concerning nanoplastics (NPs). Detection, quantification, and source identification of nanoplastics (NPs, 1-1000nm) in different matrices is still one of the largest challenges in this context due to the lack of adequate sample preparation procedures and straightforward analytical techniques for their characterization and quantification. In addition, design and fabrication of relevant reference and standard plastic particles within targeted nano-size ranges is of crucial importance for developing straightforward analytical techniques.

In the framework of the research project MS4Plastics (H2020-MSCA-IF-2020 - Grant Agreement No 101023205) different approaches such as high-energy grinding, extensive sonication, and exposure to ultraviolet (UV) light to initiate photochemical reactions leading to the breakdown of plastics were employed to degrade or fragment different plastic materials (e.g., a surgical face mask, polymer pellets, rubber dust, and plastic powders) into NPs. For detection and size determination of NPs formed after employing different approaches for plastic fragmentation dynamic light scattering was used. Finally, the possibility of using asymmetric flow field-flow fractionation (AF4) hyphenated to multi-angle light scattering (MALS) as a tool for characterization of a subset of the produced NPs samples was investigated.

### **3.14.P-Mo190 Machine Learning to help identifying polymers**

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Monitoring microplastics is important for science as well as for environmental and water authorities. Therefore, rapid and accurate detection and identification of these compounds is critical. Recent research has shown that machine learning can help to identify microplastics larger than 10  $\mu\text{m}$  reliably, using simple models such as knn and more sophisticated ones such as three different Convolutional Neural Network (CNN) models. The next step would be to test these models on smaller particles. As particles are getting smaller their infrared spectra will get more difficult to interpret. Here machine learning can be a very useful tool.

Microplastic samples were analyzed using the quantum cascade laser (QCL)-based Agilent chemical imaging LDIR system. A database with spectra from commercially available and recorded data in the laboratory was used to train the models. In a first step to increase the database, N synthetic samples per polymer class were generated on the basis of data augmentation. Specifically, we resampled the replicate spectra by (i) randomly picking two observed spectra of the same specific polymer, V1 and V2, (ii) setting two random weights, w1 and w2 (s.t.  $w1 + w2 = 1$ ), (iii) calculating the weighted sum,  $w1V1 + w2V2$ , as the new spectra, and (iv) repeating the first three steps N times.

Afterwards, two different types of models were tested using this database. Performance of the models is evaluated using three performance indicators, namely accuracy, precision and recall. Also the model training time is compared. For the knn model the numerical spectral data (cartesian coordinates) from LDIR was used as is, whereas for the CNN models the data was first transformed from cartesian coordinates into polar coordinates and afterwards into a picture.

Conclusion: The most rudimentary model can still prove effective. In our research, a basic sub-KNN model yielded superior results when compared to the three CNN models, with the added advantage of being quicker to train. The amount of high-quality samples available is vital in the model training process. Taking into account our limited dataset, the proposed data augmentation approach led to a significant enhancement in performance. While the 2D-CNN models may not be optimal for this case study, they appear to be more effective in handling multidimensional spectroscopic signals. Further research will explore their potential usefulness.

### **3.14.P-Mo191 Analysis of microplastics under 20 µm in road dust- A case study in Seoul, South Korea**

*Dana Fahad Mohamed<sup>1</sup>, Inae Jeon and Jung-Hwan Kwon, Korea University, Korea, Republic of (South)*

The widespread occurrence of microplastics (MPs) in road dust raises substantial concerns for both the environment and human health. Analyzing MPs smaller than 20 µm is crucial due to their heightened risk of ingestion, respiratory exposure, and potential transport of harmful substances. However, current methods have limitations in detecting MPs smaller than 20 µm, making the analysis time-consuming and challenging. In this study, road dust samples were collected from 30 different locations in Seoul, South Korea. An in-depth analysis of microplastics (<20 µm) in road dust using Confocal Laser Scanning Microscopy (CLSM) and Pyrolysis Gas Chromatography-Mass Spectrometry (Py-GCMS) was conducted. CLSM was used to quantify the number of plastic particles. A counter staining technique using Nile red and calcofluor white was used to exclude any false positives from the sample. The number of particles ranged from 16-1960 MP particles/mg sample. Py-GCMS was used to determine the mass of microplastics in road dust. The mass of microplastics in road dust ranged from 0.342 to 2.96 µg plastics/ sieved sample. The findings not only provide a comprehensive understanding of microplastic distribution in road dust around Seoul but also underscore the importance of employing advanced methodologies to overcome measurement limitations associated with smaller particles.

### **3.14.P-Mo192 Cascade Filtration for Multi-technique Characterization of Micro-Nanoplastics in Environmental and Human Samples**

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Micro-Nanoplastics (MNPs) are defined as plastic particles smaller than 5µm and have become ubiquitous environmental pollutants due to the widespread use of plastic materials. Their presence in various environmental compartments, including water, sediment, soil, and even the human body, raises concerns about their potential adverse effects on ecosystems and human health. However, comprehensive characterization of MNPs remains challenging due to their small size, complex composition, and the presence of natural and synthetic fibers that can interfere with their identification and quantification. To address these challenges, we have used a novel cascade filtration method using silicon (Si) and alumina (Al<sub>2</sub>O<sub>3</sub>) filters to enhance the combined characterization of MNPs in environmental and human samples. The optimized filtration system effectively captures MNPs across a broad size range while minimizing losses and cross-contamination. The Si filter effectively removes larger MNPs (larger than 800nm), while the alumina filters efficiently capture smaller MNPs (800 nm – 90nm). This sequential filtration process ensures the recovery of MNPs across a wide size range, preventing the loss of smaller MNPs that often go undetected in conventional filtration methods. The separated MNPs are then subjected to further characterization using microscopy, spectroscopy, and pyrolysis-gas chromatography/mass spectrometry techniques to gain insights into their polymer type. Cascade filtration, coupled with advanced analytical techniques, provides a powerful tool for comprehensive microplastic characterization, enabling a better understanding of their sources, distribution, and potential impacts.

### **3.14.P-Mo193 Detection of microplastic particles (1-10µm) in soil matrices**

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Quantifying the number of microplastic particles (MP) < 20 µm remains a challenge. Electron microscopy provides sufficient resolution, allows for an elemental analysis of light elements and comes with a high degree of automation. In this study, we, therefore, explored the potential of automated scanning electron microscopy (SEM) to assess MP between 1 and 10µm. We spiked Lufa 2.4. soil sieved to 0.45-10 µm with polyethylene (PE) and polyvinyl chloride (PVC) MPs of similar sizes to mimic a contaminated soil. The mixtures were suspended in 100% ethanol or isopropanol and filtered through gold-coated membrane filters (Nuclepore, Whatman). For imaging, we used a backscattered detector and the particles were detected based on their density contrast relative to the Au coating. The operational conditions (acceleration voltage of the SEM (3kV) and the thickness of the Au coating (40 nm)) were derived from Monte Carlo simulations of the interaction of the electron beam with solid materials using the software code CASINO (v. 3.3.0.4) [1]. The particle detection was automated using the software code 'feature' (Oxford Inst.). The classification of detected particles was based on their elemental signatures recorded with a windowless energy dispersive x-ray (EDX) analysis system (X-TREME, Oxford Inst.). PVC particles filtered from pure suspension sample resulted in a correct identification of 70% of all particles, whereas 15% of the particles were identified as PE and soil particles, each. In PE samples, 85% of all particles were identified as PE and 15% as soil particles and in the pure Lufa 2.4. soil sample, 93% of the particles were classified as silicates and 7% as PE. The somewhat lower detection rate of PVC is most likely due to the volatility of chlorine under the electron beam. To assess the potential of the developed method to identify different types of MPs in complex matrices, mixtures of PE, PVC and Lufa 2.4. soil in a 1:1:2 ratio were filtered on

Au coated Nuclepore filters. In total, 243 PE, 213 PVC and 508 soil particles were detected on a subset of 35 images corresponding to an area of 0.25 mm<sup>2</sup>. These particle number translate into number based ratios of PE:PVC:SOIL of 1:0.9:2.1 corresponding well to the expected ratio and demonstrate the potential of our approach.

[1]: Demers, H., et.al. (2011), Three-dimensional electron microscopy simulation with the CASINO Monte Carlo software. Scanning, 33: 135-146

### **3.14.P-Mo194 Exploring Oil-Based Extraction for Nanoplastic and Microplastic Analysis in Solid Matrices using a New Type of Reference Material**

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The analysis of nanoplastic (NP, size < 1µm) in any matrix is still challenging. Especially in solid particulate sample NP analysis is hampered by its low concentrations and a significant lack of extraction methods. In environmental samples NP is expected to be present together with microplastic (MP) loads. However, knowledge about NP and MP concentration would be important i.e. in soils, compost, sewage sludge, or filter sludge. One promising method would be the oil-based extraction (OBE) of NP due to the lipophilic properties of polymers. However, OBE was only applied to samples with limited particle sizes and no OBE of NP is reported yet.

To enhance NP analysis, we're developing precise NP and microplastic (MP) reference materials using innovative methods. These materials mimic specific characteristics, allowing controlled experimentation. We're exploring OBE adaptations by varying experimental conditions, aiming for efficient particle extraction. Extinction measurements show potential for fast NP concentration determination.

Our NP reference materials demonstrate 180 nm diameter, while our monodisperse MP materials vary between 100 and 500 µm with uniform disk-like shapes. Initial small batch experiments revealed low NP extraction rates using stirring and shaking methods, prompting investigations into more effective mixing techniques. Large batch experiments with cryo-grinded MP particles yielded a 44% recovery rate.

Challenges persist in OBE for NP and MP extraction from solid matrices, demanding optimization of process parameters and addressing analytical hurdles. However, OBE's advantages, like non-use of hazardous chemicals and size-independent efficiency, make it promising for environmental sample analysis.

Furthermore, our approach introduces a novel method for producing MP reference materials with defined properties, offering scalability and the potential for materials with fluorescence or magnetic properties. This innovation could revolutionize reference material production and properties, aiding in diverse research avenues.

### **3.14.P-Mo195 Sensitive Analysis Method for Microplastics Using Wavelength Tunable Quantum Cascade Laser and Photoacoustic Detection**

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Human activities are responsible for introducing microplastics (MPs) into freshwater and marine environments. The real impact of MPs remains largely unknown due to their slow degradation, allowing them to persist in the environment for many years. There is an urgent need to enhance our understanding and develop innovative methodologies for their real-time, rapid, and efficient detection across different matrices and environmental compartments. This is crucial for comprehending the ecotoxicological effects on different biological levels and implementing effective removal strategies.

This study proposes a novel microplastic detection method utilizing a wavelength-tunable quantum cascade laser as a light source and a photoacoustic sensor for detection. The method relies on spectrally dependent light absorption by plastics, like the well-established Fourier-transform infrared (FTIR) spectroscopy method. Aqueous dispersions of polystyrene (PS) and polyvinyl chloride (PVC) MPs sized between 25 and 70 µm were prepared in ultra-pure water, followed by manual agitation for one minute until homogenous dispersion. MPs were then extracted by filtrating the solution through a gold-coated filter. Key components of the detection system include a source of energy, a chopper that has a crucial role in modulating the laser beam, and an extremely sensitive cantilever microphone that is a highly sensitive device that measures sound and allows the

detection of very weak signals coming from the plastic particles, enabling the identification and quantification of MPs based on their unique spectral fingerprints. The wavelength range in the data is from 8.9 to 10.3  $\mu\text{m}$ , which is limited by the tuning range of the quantum cascade laser model used in this work. All the recorded spectra are compared against the reference FTIR spectra existing in the siMPLe library. The obtained data has a good correspondence with FTIR database spectra. This photoacoustic sensor offers several advantages over the already existing techniques, including high sensitivity and specificity, being able to detect and identify MPs samples containing only a few particles in the filter. This research not only addresses the urgent need for reliable MPs detection methods but is also a breakthrough for future applications in related fields such as polymer characterization and environmental impact assessment.

### **3.14.P-Mo197 Pyrolysis-Gas chromatography-Mass spectrometry : Way Forward To The Low- $\mu\text{m}$ and nm Range Plastics Analysis**

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In recent years, the microplastics and nanoplastics pollution has gained an important interest but methods to identify and quantify them still need to be standardized. The use of pyrolysis-gas chromatography/mass spectrometry for the analysis of low- $\mu\text{m}$  and nm range plastics is a promising approach as the obtained results are mass-based and not based on particles number.

This study aims to contribute to the understanding of plastics analysis by investigating the impact of particle size on the chromatographic outcomes. To achieve this, four distinct polymers, including polystyrene, polyethylene, poly(methyl methacrylate) and polypropylene, each characterized by varying particle sizes, along with a microplastics mix, were subjected to thorough analysis using Py-GC-MS. By exploring the obtained chromatograms, this research seeks to provide valuable insights into the efficacy of Py-GC-MS in elucidating the complexities of plastics at the low- $\mu\text{m}$  and nm ranges.

The analysis of different sizes, ranging from micro to nanometer, of different polymer standard particles show an impact of the particle size on the obtained chromatogram. Indeed, a change in ratios between peak areas is observed depending on the polymer size. These results will have to be taken into account while analyzing environmental samples. As such, one of the possible solutions is a size separation step during the sample preparation procedures. Another solution could be to build two different types of calibration curves or a calibration curve based on a mix of different particle sizes in order to represent as much as possible what can be found in the environment.

To conclude, due to its mass-based detection, the Py-GC-MS seems to be a interesting tool in the analysis of nanoplastics. However, extensive studies have still to be carry on nanoplastics to assess the deviation compared to microplastics in order to achieve a reliable quantification in environmental samples.

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### **3.14.P-Mo198 Microplastic Characterization and Screening by Combining DART and High-Resolution Mass Spectrometry**

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Microplastic particles result from commercial product development and the breakdown of plastics and are abundant in our environment from a variety of commercial sources like cosmetics, textiles and other pieces of plastics such as water bottles that breakdown with radiation. Microplastics have been detected at an alarming level in our marine life and drinking water. Direct Analysis in Real Time - High Resolution Mass Spectrometry (DART-HRMS) allows for rapid fingerprinting of environmental microplastics, e.g. for the identification of their origin. Typical samples of interest are polymers found in the environment like virgin pre-production pellets, microbeads from personal care products, microplastics found in the aquatic environment, and synthetic fibers.

DART was coupled to an impact II VIP QTOF (both Bruker, Germany). While traditional GC/MS methods require sample preparation (10-20 min) and long analysis times (> 20 min), this new method allows for rapid polymer analysis (< 5 min) without sample preparation. A small sample sliver is cut and placed in a copper pot of a thermal desorption system (IonRocket, Biochromato Inc., Japan) which delivers temperature gradients from ambient to 600 °C with ramp rates of 150 °C/min. When the thermal program is started, the QTOF collects MS spectra at 5Hz data acquisition speed for the entire run.

The resulting mass spectra display thousands of discrete peaks. Depending on the temperature, different additives, the polymeric basis as well as degradation products are released and detected at different time points during the run. Distinct m/z signals and general signal patterns of a microplastic material found in water were compared with standard plastic samples such as a soda bottle, a trash bag and packaging material. While the soda bottle turned out to be made of PET, the microplastic clearly had PE as a basis. PE as an insoluble polymer is difficult to analyze with other MS-based methods like MALDI. The

microplastic material showed most similarity with the trash bag, regarding both certain distinct marker m/z values as well as the general pattern released by the temperature gradient, so it can very likely be traced back to that origin.

### **3.14.P-Mo199 Additive manufacturing of 3D-printed monodisperse microplastic standard particles as an internal standard and for calibrations in microplastic analysis**

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The analysis of microplastics has made enormous progress in recent years and affects almost all environmental compartments from soil, air and water. The EU Directive (DIRECTIVE (EU) 2020/2184) obliges all EU countries to introduce a methodology for measuring the microplastic content in drinking water. For analysis it is often necessary to calibrate, approve or validate the methods with known standard material. There is also a need for microplastic particles with defined properties to investigate the behavior of microplastics in different environmental compartments, e.g. to investigate the influence of polymer type and particle size on transport properties.

So far, microplastic standards have been produced mainly by cryogenic grinding and screen fractionation. This is associated with high costs for liquid nitrogen and only leads to dispersed microplastics with a particle size distribution within the limits of the sieves used.

Monodisperse particle standards are therefore not possible. Due to the particle size distribution, the exact number of particles cannot be deduced from the mass of the fraction. If an exact number is required, this must be counted at great expense of time.

Here we present our new approach, which enables piece-count accurate additive manufacturing of single microplastic dots in the 3D printer. Monodisperse particles are deposited on a printing surface within only a few minutes. Furthermore, this method is cost-effective and can be realized with almost any thermoplastic, including biodegradable plastics.

In terms of environmental friendliness and sustainability this method has further advantages. Cryogenic grinding and screening also result in additional fractions of microplastics of other size classes. With the new manufacturing method, the excess of microplastic produced is reduced to a minimum.

A patent is pending for the process under patent number PCT/DE2023/100515. So far, particles with a size dimension of approx. 100 µm have been successfully produced. Specially manufactured nozzles with diameters of 80 down to 10 µm were tested in first polymer extrusion-tests and promise smaller particles in the future. The materials tested so far are PLA, fluorescent PLA and PCL. We currently also produce our own filament from PE, PET and blends using our own extruder system.

The microplastic standards produced are used for experiments on microplastic enrichment from sediment-rich matrices by means of electro-, density- and oil separation.

### **3.14.P-Mo200 Production and Analysis Methods for more Environmentally Relevant Small Microplastic and Nanoplastic Test materials**

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Microplastic (MP; 1-5000 µm) and nanoplastic (NP; <1 µm) particles observed in the natural environment are typically (i) irregular-shaped, (ii) partially degraded, (iii) a wide range of polymer types, (iv) a continuum of sizes, shapes and densities, and (v) a reservoir for complex mixtures of plastic-associated chemicals. There is a need for environmentally relevant MNP test materials for assessing fate and effects. Small MP (sMP; <50 µm) and NP are thought to be the highest risk size ranges, but cryomilling struggles to produce meaningful yields. Here, we present the results from an assessment and optimisation of three previously reported strategies for producing sMP and NP [1,2]. Strategy #1 used pre-cryomilling, thermal treatment, UVC irradiation and probe sonication of PS, PE and PET materials. Strategy #2 involved the testing of different mechanical degradation processes after pre-cryomilling, including bath sonication, probe sonication and wet grinding (Ultra-Turrax). Strategy #3 investigated the suitability of partial solubilisation with a long chain alkane.

Strategy #1 was viable for producing PS, PE and PET sMPs. A measurable increase in the yield of particles in the range 1-10 µm was observed, with the percentage mass yield increasing from ~0.01% to ~0.7% for PS, from ~0.5% to ~3% for PE and from ~0.1% to ~2.5% for PET. However, overall yields remained very low and there was no measurable increase in the amount of NP. Strategy #2 resulted in no change in the average particle size (z-ave) for cryomilled PE under any of the treatments, with high PDIs indicating a broad particle size distribution. Some reduction in z-ave was observed for cryomilled PET, with probe sonication and wet grinding the most effective treatments, reducing the z-ave from ~6000 nm to ~1800 nm and ~2000 nm, respectively. The PDI was correspondingly reduced, but there was no increase in the proportion of NPs.



Strategy #3 resulted in PE particles with z-ave of ~650 nm and a high PDI. The z-ave of PET, PA, PAN and wool ranged from ~200-300 nm, with reduced PDI values suggesting a narrower particle size distribution. Full physicochemical characterisation is currently being completed. The alternative top-down methods evaluated in this study for producing environmentally relevant SMP and NP test materials show potential. Partial solubilisation appears promising for producing, but more characterisation of the resulting materials is needed to confirm their environmental relevance.

### 3.14.P-Mo201 Multi-modal Imaging as a Tool to Decipher the Biological Response of Nanoplastic Exposure

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Since the conception of plastic polymers in the 1950s, plastic production has skyrocketed to meet the ever-increasing demand. With estimates of over 14 million tons of microplastics (MPs) in the environment, the fragmentation of larger plastics into MPs is widely understood. However, the further fragmentation of MPs into nanoplastics (NPs, dimensions < 1µm) presents a new area of study in the field. In fact, NPs have been said to be one of the most hazardous components of marine litter. As a direct result, there has been a steady increase in scientific interest pertaining to the potential adverse effects of NPs. The inherent analytical challenges of identifying carbon-based polymers in biological matrices necessitate a novel suite of analytical methods. The use of multi-modal imaging techniques unlocks the capability of co-locating NPs and their associated biological effects, furthering our understanding of the biological response triggered by these NPs. Ultimately, we aim to investigate not only the distribution and biological effects of NPs but also adsorbed co-contaminants on NPs, evaluating the capacity of NPs as a vector for other legacy Persistent Organic Pollutants (POPs).

Here, we developed a spatially resolved omics analysis using Particle-Induced X-ray Emission Spectroscopy and Matrix-Assisted Laser-Induced Desorption Ionization Mass Spectrometry. By doing so, we aim to identify chemical changes in the tissues surrounding plastic particles. Zebrafish (*Danio Rerio*) embryos were exposed to nano-polystyrene (*nPS*) at environmentally realistic concentrations via media and microinjection. The embryos were euthanized at 4 days post-fertilization, sectioned to 20µm, and analyzed. Changes in elemental and chemical distribution were observed in several vital organs, including the heart. This study provides insight into the biological effects of NPs and paves the way for more focused studies on the specific mechanisms triggered by NPs. Additionally, the potential impact of this research extends to informing environmental policies and practices, contributing to the development of sustainable solutions for managing plastic pollution in aquatic ecosystems. There is ongoing research to couple this analytical workflow with intrinsically tracking the exposed NPs in biological tissue.

### 3.14.P-Mo202 Investigating (Hetero-) Agglomeration of Nanoplastics using Particle Tracking Analysis

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A comprehensive analysis of nanoplastics (plastic particles < 1 µm) in the environment usually requires many different properties of these particles to be characterized. This makes it a challenging task with different techniques that must be employed. With regards to this, also fate and transport of nanoplastics must be understood well for representative sampling. One major factor influencing the transport properties of such particles is (hetero-) agglomeration. The interaction of nanoplastics especially with inorganic particles found in the environment can play a critical role in their fate and transport behavior. Particle Tracking Analysis (PTA) is a light scattering based technique to gain information on individual particles and agglomerates. It can be used to study particle size and concentration over time and thus indicate agglomeration. Additionally, the zeta potential can be characterized as the surface charge plays an important role in the interaction of particles. For strongly agglomerating samples cryo-scanning electron microscopy (SEM) can be used for further characterization. Conventional sample preparation for SEM has the disadvantage that particles start agglomerating during the drying process. Thus, the suspension is frozen using liquid ethane and then analyzed at low temperatures to maintain the structure of the sample. In this study, a wide size range of plastic particles (60 – 600 nm, including polystyrene, polyethylene and poly(methyl methacrylate)) will be employed and mixed with inorganic particles consisting of TiO<sub>2</sub>, SiO<sub>2</sub> and iron oxide. In a further step, organic substances, like humic acids and proteins, can be added to evaluate their influence on particle agglomeration in a controlled matrix. At last, an environmental sample will be spiked with plastics and their agglomeration will be investigated. In further studies PTA can be online-coupled to separation techniques like field flow fractionation (FFF) to achieve even higher resolution over a broader size range and more detailed information on complex mixtures. In hyphenation with Raman microspectroscopy also chemical information about suspensions could be achieved. Overall, a more detailed insight into particle agglomeration of nanoplastics will greatly benefit the understanding of transport and fate of this complex analyte. This would not only improve sample preparation for quantitative measurements, but also support toxicity studies and targeted measures to remove these particles.

### 3.14.P-Mo203 µ-Raman analysis of a candidate microplastics' reference material – a proof of concept

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Microplastics (MPs, synthetic polymer fragments in the size range 1 µm–5 mm) are found in the environment all around the

globe (in the water, soil, biota, etc.) as well as in drinking water and food, thus raising concerns about their impacts on the environment and human health. Therefore, reliable size-resolved identification and quantification of MPs in different samples, that can be only achieved by application of validated methods is needed. However, validation of analytical methods for measurements of MPs is currently hampered because of a general lack of reference materials.

To tackle this problem, a candidate reference material has been developed by EC JRC, consisting of polyethylene terephthalate (PET) particles and a water matrix. It is based on the concept presented elsewhere (PET particles embedded in a layer of NaCl, which must be reconstituted into water using a surfactant solution), but with a low PET particle number concentration. According to the definition, RM must be sufficiently homogeneous and stable with respect to one or more specified properties. In case of this analyte (PET particles of a defined size range), the analytical method needed for the assessment, to comprise the automated counting of particles, their size determination and confirmation of chemical identity. This has been done, for the first time, by Raman microspectroscopy ( $\mu$ -Raman) combined with a tailor-made software. Several units of the candidate RM allocated to homogeneity, stability and verification of a small size particle content were analysed by  $\mu$ -Raman. This technique is a suitable tool for the chemical identification and quantification of MPs down to 1  $\mu$ m. Automated  $\mu$ -Raman analysis has been performed using recently developed open-source program *TUM-ParticleTyper 2*. The latter enables the automated detection, quantification, and morphological characterization of (plastic) fragments in images of optical microscopy, followed by the automated  $\mu$ -Raman-based identification of MPs and non-plastic fragments. This approach allows for analysis of up to 7000 particles down to 10  $\mu$ m, randomly selected on the entire filter. For the analysis of particles down to 1  $\mu$ m an improved strategy, including subsampling with random windows, and a bootstrap method for estimating the quantification error was used. In this presentation we will discuss a suitability of the applied approach and the tailor-made software to fully evaluate a candidate RM with microplastics as a proof of concept.

### **3.14.P-Mo204 A Chemometric Approach to Improve the Identification and Quantitation of Plastic Particles in Human Blood Using Pyrolysis-GC-MS**

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Accurate quantitative methods are crucial to assess human exposure to micro and nano plastics (MNPs). Quantitative pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) has recently been used for quantifying MNPs in human blood. However, pyrolysis introduces complex effects, including potential secondary reactions between matrix compounds and polymers, impacting current quantification methods based on single markers.

This study uses a chemometric approach to enhance the identification and quantification of MNPs in human blood. A non-targeted approach was used to investigate all thermal degradation products and study their behaviour. Blood samples spiked with MNPs were analysed to identify effective markers for various polymer types while addressing co-pyrolysis and matrix effects. Comprehensive multivariate analysis is first applied to polymer standards' reference data and then extended to an in-matrix study.

Based on the method by Leslie et al. (2022), human whole blood was spiked with varying PET, PE, and PVC concentrations and was analysed using Py-GC-MS. Utilizing the PARAFAC2 algorithm in PARADISE, data was extracted for an untargeted analysis of all pyrolysis products. Multivariate quantification, employing random forest and PLS-regression was compared to single-variable linear regression.

The multivariate quantification (using 416 features) outperformed single-variable regression, especially for PVC analysis. Recursive Feature Elimination was used to select a subset of optimal features and showed more than 15% decrease in quantification error compared to single-marker quantification. The pyrolysis of PE and PVC is identical in blood, while PET appears to exhibit complex interactions with the matrix, resulting in newly formed compounds and expected markers being reduced. However, the affected compounds are able to quantification PET, while reproducibility can be doubted. Additionally, the presence of PE appears to increase PAH formation during the pyrolysis of PVC, causing overestimations in PVC concentrations.

In conclusion, this study highlights the importance of considering secondary effects in pyrolysis, proving the value of a multivariate approach for the identification and quantification of MNPs in blood. Markers are identified, and the importance of understanding potential interferences and secondary reactions is highlighted, emphasizing awareness of the present polymer types and matrix compounds during pyrolysis.

### **3.14.P-Mo205 Human Exposure to Airborne Micro and Nano Plastics in Indoor Areas**

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Background: Micro and nano plastics (M/NPs) are even detected in remote areas, raising significant health concerns. While much research focused on marine and soil environments, the underestimated threat of exposure to airborne M/NPs (aM/NPs) is

concerning. Their low density and small size, pose potential health risks through inhalation and ingestion. Accurate sampling, measurement, and analysis of aM/NPs are complex challenges that require a critical evaluation of existing techniques. Consequently, we conducted a trial experiment to assess indoor aM/NP exposure, considering human activity intensity.

**Materials and Methods:** MP deposition samples were collected from 3 types of rooms in a laboratory in Leuven, Belgium using glass funnels attached to Erlenmeyer flasks over 10 working days. This experiment was conducted during 4 different periods: Low activity/Cold season, High activity/Cold season, Low activity/Warm season, and High activity/Warm season. Environmental factors including CO<sub>2</sub> concentration, flow rate, relative humidity, and temperature were measured. Macro imaging and ImageJ software were used for quantification purposes, while morphological characteristics were analyzed with a stereomicroscope. For chemical identification reference materials were used for optimization of qualitative analysis by  $\mu$ -Raman, Pyro-GC/MS, and high spectral imaging and then the chemical characteristics of the collected sample will be done using these techniques.

**Results and Discussion:** Our prior study highlighted the importance of careful consideration in five critical steps: pre-sampling, sampling, post-sampling, analysis, and contamination control, ensuring precise results for human exposure to aM/NPs. Our findings consistently showed the highest M/NP concentrations in the kitchen and dining area across all seasons, with notably higher counts during the cold season. During this season, human activity primarily contributed to aM/NP levels in offices, kitchens, and dining areas, while in the warm season, external sources, along with human activity, contributed to increased particle counts. Laboratories consistently experienced primary exposure from human activity.

**Conclusions:** A comprehensive assessment of aM/NPs necessitates diverse techniques and contamination control measures. Human exposure to particles is influenced by factors such as human activity, cleaning schedules, and air quality.

### **3.14.P-Mo206 A Novel Sampling Approach for Airborne Microplastics: In Situ Sampling and Extraction**

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Many analytical difficulties are encountered when determining micro/nano plastics in air using conventional atmospheric particulate matter sampling methods such as high-volume air samplers or impactors. The small size and very large number of microplastics (MPs) in the air make it difficult to use extraction methods used in the literature for other environmental matrices in air samples. The aim of this study is to design and develop a novel sampler to collect and extract airborne MPs from atmospheric particles in situ. For this purpose, two separation funnels were connected in series to an air pump. While atmospheric particles settled to the bottom of the separation liquid due to their higher density and coarse structure, airborne MPs were collected on the surface of the separation liquid due to their lower density. Two salt solutions with different densities (NaCl: 1.2 g/cm<sup>3</sup> and ZnCl<sub>2</sub>: 1.6 g/cm<sup>3</sup>) in all possible combinations were put in separation funnels connected in series for in situ sampling of MPs from the air. The air was sucked with a flow rate of 5 L/dk, and both fractions were filtered onto a Polycarbonate filter and then visually analyzed using a stereomicroscope. According to preliminary findings, the percentage of total MP collected on the solution surfaces was for NaCl-NaCl, NaCl-ZnCl<sub>2</sub>, ZnCl<sub>2</sub>-NaCl, and ZnCl<sub>2</sub>-ZnCl<sub>2</sub> combinations, 75.69%, 51.28%, 81.56%, and 85.80%, respectively. Despite some minor settling of MPs, the presence of ZnCl<sub>2</sub> solution in both separation funnels proved to be more effective in collecting and separating than other alternatives. Fibers were the most prevalent form of MPs in all samples and the most abundant MPs were observed in blue, transparent, and yellow colors, with the most abundant range of MPs were 100-500  $\mu$ m. This new MPs sampler presents a practical approach for the efficient collection of airborne MPs and a pioneering idea to avoid positive and negative contamination from pre-treatments.

**Keywords:** Active sampling, Airborne microplastics, Method development, Novel sampler for MPs

### **3.14.P-Mo207 Impact of Microplastic Air Pollution From Intensive Agriculture in South-Eastern Spain**

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The atmosphere is currently recognized as a major vector of long-distance microplastics (MPs) transport. The major sources of airborne MPs are the synthetic clothing, furniture, building materials, abrasion of rubber tires and city dust. However, the knowledge about the concentrations of airborne MPs worldwide is still limited, due to the lack of data and standardized protocol for their extraction and quantification.

On the other hand, during the last 20 years, the production of vegetables in a protected environment has contributed importantly to the national economies in Mediterranean regions, especially in the Spanish province of Almería, which has the largest area covered by greenhouses in the world. The construction of greenhouses requires the use of many synthetic materials, which are used for roofing, mulching, ropes, and clips to hold plants, among others. This has led to the production of large amounts of plastic residue. Thus, the main objective of this work was evaluating the impact of plastic pollution due to intensive agriculture in this area.

For that, a MP passive sampler was developed to give a more comprehensive information about the presence of MPs in the atmosphere around greenhouses. The device consisted of a funnel constructed by a 3D printer containing a stainless-steel filter

of 25 µm and 30 cm of diameter. A continuous record of the air flow adapter to a flowmeter provided an accurate estimation of the concentration of the MPs per m<sup>3</sup>. Air samples were taken inside and outside a greenhouse, as well as at an altitude of 100 m to compare the extent to which intensive greenhouse agriculture affects to the airborne MP content. A stereomicroscope and a micro-FTIR were used to identify the number of MPs and classify them in terms of morphology, color, and type.

The results revealed that the number of plastic particles was lower at 100 m height than at ground level (36 items/m<sup>3</sup> and 70 items/m<sup>3</sup>, respectively). The average total area of MPs was around 140 µm<sup>2</sup>/m<sup>3</sup> at 100 m altitude and 310 µm<sup>2</sup>/m<sup>3</sup> at greenhouse level. Fragments were the most detected particles, followed by fibres, and films. The main colours found were blue, black and red. Up to a total of 9 different synthetic polymers were identified by FTIR analysis. PP, PA, and PE were the most frequent polymers detected in the samples analysed.

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### **3.14.P-Mo208 Arctic Airborne Pollution: Tracking Microplastics and Plastic-Associated Contaminants in Canadian Snowfall**

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Plastic pollution produces adverse impacts caused by plastic pieces, additives, and chemicals adsorbed on plastics in the environment. Atmospheric deposition, particularly through snowfall, facilitates the infiltration of airborne microplastics (1 µm - 5 mm) and plastic-associated contaminants into soil and aquatic ecosystems. However, little is known about how microplastics and plastic-associated contaminants deposit into the Canadian Arctic via atmospheric deposition. Therefore, this study investigated the occurrence and distribution of microplastics and three groups of plastic-associated contaminants: organophosphate esters (OPEs), per- and poly-fluoroalkyl substances (PFASs), and benzotriazole UV stabilizers (BZT-UVs) in Canadian Arctic snow. Samples were collected between February 2020 and May 2021 from Little Fox Lake (Yukon; n=6), Yellowknife (Northwest Territories; n=5), Cornwallis Island (Nunavut; n=9), and Alert (Nunavut; n=15). We employed filtration (>20 µm), microscopic visualization, and FTIR for microplastic analysis. Plastic-associated contaminants were extracted by solid phase extraction and analyzed by liquid chromatography-tandem mass spectrometry or gas chromatography coupled to triple quadrupole mass spectrometry. The microplastic abundances were as follows: Cornwallis Island (50 ± 10 n/L) (counts per L, mean ± S.E.), Alert (25 ± 7 n/L), Little Fox Lake (17 ± 8 n/L), and Yellowknife (12 ± 3 n/L). Prevalent shapes included fibers (48%), fragments (42%), and films (9%), with an average length of 695 ± 56 µm (mean ± S.E.). Analysis revealed 22 plastic polymers, with polypropylene (30%) and polyester (25%) as dominant. Microplastic deposition flux estimated at Alert was 57±9 microplastics/day/m<sup>2</sup> between February 2020 and May 2020. Yellowknife and Cornwallis Island samples were analyzed for OPEs and PFASs. OPEs and PFASs were frequently detected (>75%) in the samples, with a mean concentration of Σ<sub>26</sub>OPEs 97 ± 5 ng/L and Σ<sub>23</sub>PFASs 3.5 ± 0.3 ng/L. Preliminary findings showed a negative correlation between total microplastics and dissolved triethyl phosphate concentration, but no correlation was observed with other analyzed OPEs or PFASs. The analysis of BZT-UVs is currently underway. Upon completion of data analysis, our study will examine relationships among microplastics, OPEs, PFASs, and BZT-UVs, increasing baseline knowledge on the transport of microplastics and plastic-associated contaminants into the Canadian Arctic.

### **3.14.P-Mo209 Small microplastics (<100 µm) in the urban atmosphere: a comparison between aerosol and wet-dry depositions**

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MPs (microplastics) have been found widely in terrestrial to aquatic ecosystems; however, only recently has greater scientific attention been focused on the presence of MPs in the atmosphere and their potential role in human health implications. Hence, the atmosphere is considered one of the most important pathways for the transport of emerging contaminants, including the smallest size of MPs (SMPs <100 µm), since they are easy to be transported through the air circulation and inhaled by humans and biota. However, the SMP fluxes from aerosol to the ground due to the wet and dry depositions are still unknown. In our study, we provide an entire SMP dataset for aerosol and wet/dry depositions collected in an urban area to understand better their fluxes in an urban atmospheric system, employing the same methodology with a QA/QC (Quality assurance and quality control) protocol. Aerosol and wet/dry deposition samples were collected in three different campaigns from winter 2021 to winter 2022 near Venice, Italy. The sampling was carried out on the roof of the scientific campus of Ca' Foscari University of Venice in Mestre City. Then, a pre-treatment procedure for each matrix was performed using an oleo-extraction method and a quantification and chemical identification of SMPs was carried out using a MicroFTIR. The SMP's complete database of abundance, weight, aspect ratio, polymer typology and size was obtained for each matrix in different seasons employing the

same method. Different urban source inputs involved in the atmosphere were hypothesized analyzing the polymer typologies in each matrix. Regarding the seasonality evaluation trend, the SMPs occurrence was always higher in winter periods than in summer for both aerosol and wet/dry depositions. The majority of SMPs were confirmed to be less than 100 µm. Further, it was observed a decrease in airborne SMP abundance after each rainfall event. Hence, comparing wet and dry depositions, the most contribution derives properly from wet deposition, confirming their potential role in SMP removal due to the scavenging effect, greater than in dry periods. Also, even precipitation with a low intensity would facilitate the deposition of airborne SMPs from the atmosphere by wet depositions. Hence, the contribution of SMPs in the atmosphere was confirmed to be an important pathway that contributes to their transport in terrestrial and aquatic compartments, with potential implications for all ecosystems.

### 3.14.P-Mo210 Control of Microplastic Pollution Through Beehive Colonies

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Recent scientific papers have reported on the usefulness of honeybee hives as biological samplers for xenobiotic pollutants monitoring such as pesticides as they bring back these pollutants to the hive from forage areas. However, their use for the control of microplastics (MPs) has not yet been addressed.

In this study, several modes of MPs sampling, such as bees and pollen, have been evaluated together with a novel in-hive passive sampler, called APITrap (Apiarian-Trap). API-Trap basically consist of a rigid plastic polyethylene with a polyvinyl acetate adhesive, protected by a metallic net of 2 mm, where the MPs present in the hive are trapped. The evaluation of the different sampling modes for MPS were performed on groups of 25 bees, 2 grams of pollen, and APITrap device after two weeks placed inside the hive. Four surveillance programmes were carried out in five different apiaries in Denmark. The samples were analysed by stereomicroscopy for identifying the number of MPs and classify them in terms of morphology, color, and type (fibre, fragment, film). After morphological identification, a micro-FTIR was used to classify and identify the polymer types.

The results demonstrated a better reproducibility of the APITrap sampler compared to the analysis of honeybee and pollen samples. The average number of MPs detected over four monitoring studies ranged from 39 to 79 items in APITrap, from 6 to 9 in bees and from 1 to 4 in pollen samples. Fibres were the most detected shape, with mean detection frequency of 92%, followed by fragments (~5%) and films (~3%) in APITrap. Black, blue, green and red were the main colours of the MPs detected. FTIR analysis confirmed the presence of up to seven different synthetic polymers, been the most frequent polyethylene terephthalate (PET), followed by polypropylene (PP), polyethylene (PE) and polyacrylonitrile (PAN).

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### 3.14.P-Mo211 Tracking Urban Sources of Microplastic Contamination: Insights From the City of Modena, Italy

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Urban settings are densely populated areas that may act as a significant source of microplastics (MP, < 5 mm), such as tire wear particles (TWP) and textile microfibers. Notwithstanding, evidence on airborne MP in urban outdoor environments is confined to a handful of reports, limiting the application of MP dispersion modelling as well as environmental risk assessment. This study aims at investigating the occurrence and small-scale transport of airborne MP in Modena, a model mid-sized city located in the Po Valley (Italy).

Active and passive sampling was conducted at two sites (comparing traffic vs background conditions), during wintertime and summer. In the two periods, daily active measurements were taken for 1 week using low volume active pumped samplers (size cut-off <10µm). Sub-samples (10x10mm<sup>2</sup>) of the active filters were examined using different techniques as Scanning Electron Microscopy Energy Dispersive X-ray (SEM-EDX). Passive deposition samples were collected using stainless steel funnels equipped with glass bottles. After 14 days, passive samples were concentrated and treated to remove organic matter. Finally, samples deposited onto glass-fiber filters were examined under optical digital microscope for semi-automatic MP count and measurement. Sub-samples were further analysed by Raman microscopy (µRaman) for the polymer identification. In parallel to MP analysis, atmospheric dispersion simulations of tire wear were conducted at the city scale to estimate the spatial distribution of TWP concentrations and deposition.

In active samples, SEM-EDX confirmed the contribution of TWP in the PM<sub>10</sub> fraction in Modena. Likewise, in passive samples, a prevalence of TWP was found. Other MP retrieved from passive samples in the winter campaign mainly included fibers (76%) and fragments (34%). µRaman showed that petroleum-based and (semi)synthetic textile polymers dominated the MP cont. Other urban emission sources identified were building materials, as shown by the presence of varnish fragments. As 26% of the initial MP candidates resulted of cellulose, further processing of passive samples resulted crucial to minimize

matrix effects at  $\mu$ Raman. Ongoing analyses on active and passive samples from the summer campaign will allow to compare MP deposition rates and abundances at the two sites and time periods. Findings from the MP dispersion modelling will inform strategies to reduce MP emissions in urban environments.

### **3.14.P-Mo212 Transfer of Micro- and Nanoplastics via Sea Spray Aerosols and Estimate of Human Exposure**

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Little information is available on the possibilities of micro- and nanoplastics (MNPs) to be introduced in the atmosphere via sea spray aerosols (SSAs) and more specifically about the effects plastic characteristics have on this aerosolization process. Currently, the importance of the transport of MNPs via SSAs as a possible new exposure route for human health is unknown. Therefore, the aim of this explorative study was two-fold: (1) to examine if the polymer types, sizes and concentrations of MNPs affect the aerosolization process, (2) to perform a preliminary exposure assessment for humans based on the results of the aforementioned experiment.

This study used a laboratory-based bubble bursting mechanism, simulating the aerosolization process in the sea. Four different series of experiments were set up, to study the influence of plastic size, polymer type, plastic concentration and seawater characteristics on the aerosolization process. To determine the potential human exposure to microplastics via inhalation of SSAs, the results of the experiments in the lab were extrapolated to the field using the concentration of plastics in the sea and the volume of inhaled aerosols.

Our results indicate an enrichment of MNPs in SSAs compared to surface and bulk seawater. Aerosolization increased with decreasing plastic size and with decreasing concentration, and was higher for the higher density polymer type. Besides, the use of surface seawater instead of bulk seawater seemed to influence the aerosolization process. Our human exposure estimate shows that in comparison with reported inhaled concentrations in urban and indoor environments, this exposure route seems negligible for microplastics. Following the business as usual scenario on plastic pollution, the daily inhalation in 2100 is estimated to increase but still be far below 1 particle per day. A variation in exposure concentration can be seen due to weather conditions influencing the aerosol formation. Currently, due to the lack of environmental concentrations of nanoplastics, it is impossible to do a similar extrapolation for nanoplastics.

This study shows that aerosolization is a new plastic transport pathway to take into account, but seems negligible for microplastics in terms of human exposure. An estimation is needed for nanoplastics as a higher aerosolization and a higher exposure is expected.

### **3.14.P-Mo213 Having Fun and Raising Awareness: Italian Students Monitor Airborne Microplastic in Indoor and Outdoor School Environments**

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Densely populated areas are a significant source of microplastics (MP, < 5 mm), for example considering tire wear particles and textile fibres, easily dispersed through the air. However, public perception on MP as an environmental issue is still confined mostly to seas and oceans. Thus, while it is urgent to increase MP monitoring effort in urban environments to estimate MP levels and identify major sources and pathways, it is also important to inform the public about our own role in spreading MP contamination. To fulfil these objectives, we present the activities carried out by the University of Modena and Reggio Emilia (UNIMORE) at a local scale, which allowed to both collect scientific data on airborne MP in urban environments and raise awareness among young people.

The perception, knowledge and attitude of the public on MP contamination was initially assessed through the administration of a voluntary-based online survey (October 2022) in the area of Modena (Italy), involving young people, firms, academia and environmental associations. The educational program «Taking microplastics into the class» was then launched in collaboration with the scientific high school in Applied science in Modena to collect data on MP passive deposition in indoor and outdoor sites at the school, estimate MP exposure in high school students and promote sustainable and pro-environmental behaviours. The program included seminars about plastic pollution and an active participation of the students in MP monitoring at school through: clean-up activities, air passive sampling, sample processing (filtration) and analysis (MP count and classification using ImageJ) and presentation of the results. UNIMORE researchers coordinated field and laboratory activities, stressing the importance of quality control measures (using field and laboratory blanks) and performing additional analyses when needed (e.g., sample oxidative treatment, acquiring MP images and identifying polymers through Micro-Raman spectroscopy).

In the questionnaire, 92.8% of the 523 respondents expressed high level of concern about MP contamination, but a lack of knowledge about MP sources in urban areas was found. The activities carried out in the high school allowed to collect first

data on MP level, types and polymers found in indoor (1 site) and outdoor (2 sites) school environments, in comparison, and promoting public awareness in young citizens about the manifold MP sources and their transport in urban environments.

**3.14.P-Mo214 Atmospheric Microplastic in the Arctic and Mainland Norway; occurrence, composition and sources**  
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The majority of studies on the transport of microplastics to the Arctic have focused on ocean pathways. Ocean currents originating in the south of Europe have been proposed to function as major transport routes, carrying microplastics from the more densely populated southern areas in Europe to the Arctic (Cózar et al., 2017; Tekman et al. 2020). However, given the limited empirical data and lack of harmonized methodologies for sample collection, it is not yet possible to estimate the magnitude, composition and sources of atmospheric microplastics transported to the Arctic.

Here we present the outcomes of a study applying passive and active air-samplers for wet and dry deposition on two remote monitoring stations, Ny Ålesund (Svalbard) in the High Norwegian Arctic, and at Birkenes in mainland Norway in 2022 and 2023. We complement the results with active airsamples collected on cruises along the East- and Westcoast of Svalbard in 2021 and 2023, representing Arctic offshore samples.

All samples were handled under strict QA/QC requirements, with all sample treatment occurring in controlled conditions of clean rooms and laminar flow cabinets. After filtration on a GF/F filter, polymer determination was performed by pyr-GC/MS. The analytical method, adapted from Ishimura et al. and Matsueda et al., uses calcium carbonate as a catalyst for the simultaneous quantification of 10 different polymers and SBR rubber, originating from tires. With this adapted method it is possible to quantify PMMA (poly(methyl methacrylate)), Nylon-6 (polycapromamide), Nylon-6,6 (poly(hexamethylene adipamide)), PP (polypropylene), PVC (poly(vinyl chloride)), PC (polycarbonate), PET (poly(ethylene terephthalate)), PE (polyethylene), ABS (acetonitrile-butadiene-styrene copolymer) as well as PS (polystyrene) and PTFE (polytetrafluoroethene). All samples were accompanied with field and procedural blanks.

Results were further analysed with respect to their spatial origin and long-range transport using the Lagrangian particle dispersion model FLEXPART. Rubber from car tires and Nylon dominated most samples, followed by PMMA and PVC. The estimated concentrations were fitting well on most timepoints, with some underestimation, indicating some missing sources in the model.

**3.14.P-Mo215 Atmospheric Pathways: A Global Comprehensive Study on Microplastic Deposition**  
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Microplastic contamination is a pressing environmental concern with repercussions for ecosystems and human well-being. This study conducts a thorough analysis of atmospheric microplastic (MP) deposition, providing valuable insights into the dynamics of this emerging pollutant. While aquatic compartments have been extensively studied for MPs, their presence in the atmosphere remains underexplored. This research aims to bridge this gap by evaluating atmospheric MPs, enhancing our understanding of their distribution and transport. The main objective is to assess the characteristics and prevalence of MPs in the atmosphere in North Wales, UK. Concurrently, the study identifies MPs in the soil, facilitating a comprehensive comparison between the two environments. This analysis contributes to conclusions about potential atmospheric MP deposition onto the soil, offering insights into their transport dynamics. The sampling approach involves collecting both rainfall and soil samples over a year, with fluorescence microscopy assessing the quantity, shape, and size of MPs, and Laser Direct Infrared Imaging (LDIR) identifying their polymer composition. Our preliminary findings indicated a notable prevalence of small MPs (20-40 microns), with the abundance diminishing as the MP size increased. The temporal variations of MPs primarily aligned with the rainfall pattern, though wind emerged as a crucial factor during periods of low-intensity precipitation. In addition, the presence of MPs in the soil is anticipated to be primarily affected by vegetation coverage, and the deposition is expected to rise with increased precipitation. This comprehensive examination enhances our understanding of the environmental fate of MPs, advancing knowledge of atmospheric MP pollution and emphasizing the need for a thorough approach. In addition, this research involves global partnerships (China, Vietnam, Egypt, India, and Sri Lanka) to enable the comparison of atmospheric MP deposition across different climate zones. This collaborative effort expands the study's scope, allowing for insights into how atmospheric MPs vary globally. By fostering international cooperation, the research aims to contribute valuable data for a more nuanced understanding of the impact of atmospheric MPs on diverse environments.

**3.14.P-Mo216 Human exposure to airborne microplastics in Australian indoor air**  
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People spend more than 90% of their time indoors, where they can be exposed to atmospheric microplastics (AMPs). Despite the importance of indoor air quality, little is known about human exposure to AMPs in indoor environments. Hence, it is

crucial to identify and characterize AMPs in indoor air to understand possible human exposure levels and any associated health impacts. This study used an active sampling approach to collect AMPs (20 – 5000  $\mu\text{m}$ ) from seven different indoor locations across Southeast Queensland, Australia, where people spend most of their time. The annual human exposure levels to AMPs were calculated using scenario-specific activity levels. These activity levels were used as human exposure to AMPs varies among individuals as they spend their time in different indoor locations with different activity levels and experience different inhalation rates. The results revealed that a childcare site had the highest AMP concentration ( $2.25 \pm 0.38$  particles/ $\text{m}^3$ ), followed by an office ( $1.20 \pm 0.14$  particles/ $\text{m}^3$ ) and a school. Of the selected indoor locations, the vehicle had the lowest indoor MP concentration at  $0.20 \pm 0.20$  particles/ $\text{m}^3$ . 98% of AMPs were fibers ranging from 71 to 4950  $\mu\text{m}$  in length, and the remaining were fragments. Polyethylene terephthalate (PET) was the most prominent polymer type found across different sites. Our findings also showed that males aged 18 to 64 had the highest AMP exposure at  $3187 \pm 594$  particles/year, followed by males  $\geq 65$  years. The females between the ages of 5 and 17 obtained the lowest exposure of  $1928 \pm 549$  particles/year. This study is the first to report AMP concentrations in Australian indoor locations where people spend most of their time. Our findings highlight direct human exposure to MPs via indoor air. Further research should evaluate possible human health risks upon inhalation of AMPs.

Keywords: Active Sampling; Atmosphere Microplastics; Indoor Air

### **3.14.P-Mo217 Extraction of Microplastics at High Temperature and Pressure for Subsequent Analysis of Atmospheric Aerosol by Pyrolysis Gas Chromatography–Mass Spectrometry**

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Microplastics (MPs) are emerging pollutants of great concern since they are widely distributed in the environment. Nevertheless, their occurrence, concentration and distribution in the atmospheric aerosol is understudied.

One of the main issues related to the identification and quantification of MPs in the environment is the absence of standard methods, neither for the instrumental analysis nor for the pre-treatment procedures. Specifically, pre-treatment is crucial to produce a suitable sample to undergo subsequent analysis. The analysis of MPs by pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) has the advantage of not requiring the maintenance of the morphological characteristics of ambient MPs during the pre-treatment. A possible way for isolating MPs from aerosol filters could be an extraction with organic solvents at high temperatures and pressure. This technique has been proposed by a few studies but has not been deeply investigated.

The aim of this work is to assess the feasibility of solvent extraction as a pre-treatment procedure for the analysis of MPs in atmospheric aerosol by using Py-GC/MS, avoiding the problem of the low solubility of plastics by reaching high temperatures (about 180°C).

The accelerated solvent extractor Thermo ASE 350 was used in this work. The extraction was conducted in dichloromethane, varying the extraction parameters, such as the extraction temperature, the static time, and the number of extraction cycles, to get information about their effect – alone or interacting with each other – on the extraction yield of plastics.

The preliminary results obtained with standard polyethylene showed a higher effect of the temperature on the extraction yield than of the other parameters; the static time seemed not to produce a yield enhancement, while the effect of the cycle number is strictly related to the other parameters (i.e. the effect of cycle number is higher at low temperatures, nearly absent at high temperatures). These results suggested that it is not necessary to conduct the extraction for a long time or with a high number of cycles - with subsequent solvent consumption and waste production - to get a satisfying yield, but a fast extraction at high temperatures may be preferable.

These findings shed light on which could be the best conditions for extracting microplastics with organic solvents and contribute to the development of a method for MP analysis in the atmospheric aerosol by using Py-GC/MS.

### **3.14.P-Mo218 Assessment of the impact of local human activity on microplastic atmospheric deposition**

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The study of microplastic transfer in the atmospheric compartment is gaining traction as a scientific field, as it is likely to represent a major transport vector for microplastics into the environment. In this work, atmospheric deposition of microplastics was monitored following the same methodology at four different sampling sites characterized by varying levels and types of human activity. Two sampling sites were located in urban environments, while the other two were surrounded by agricultural activities. Two sampling sites were close to the Paris urban area and were monitored for 5 months, while the other two were close to the metropolis of Nantes, west of France and were monitored for 12 months.



After collection, all samples underwent a treatment consisting of a density-based separation and an oxidative digestion, before they were analysed using an automated  $\mu$ FTIR imaging analysis with a Nicolet iN10 by Thermo Scientific, identifying particles down to a size of 25  $\mu\text{m}$ .

Median microplastic deposition rates of 60.2 and 29.2  $\text{MP m}^{-2} \text{d}^{-1}$  were measured in the urban sites, against medians of 40.3 and 14.3  $\text{MP m}^{-2} \text{d}^{-1}$  in the rural sites. In each metropolitan area, lower deposition rates were measured at rural sites than at urban sites, regardless of the sampling period. The polymer types were similar between sites, dominated by polypropylene, polyethylene and polystyrene. Smaller particles represented the majority of the identified microplastics. Differences were however noted between sites, with PE more present in rural sites and a higher diversity of microplastics in the urban sites.

No clear impact of precipitations or wind direction on microplastic deposition was found, suggesting the differences in human activity were the dominant factor.

### **3.14.P-Mo219 Microplastics Reference Materials: Advancing Environmental Monitoring and Research**

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Microplastic pollution has emerged as a global environmental concern, posing potential risks to human health and ecosystems. Accurate and reliable analytical methods are essential for assessing the extent, fate, and effects of microplastic contamination in the environment. Microplastics reference materials play a crucial role in ensuring the quality and consistency of analytical measurements, enabling researchers to confidently evaluate microplastic concentrations and compare results across studies.

Chiron, a leading provider of microplastics reference materials, offers a comprehensive range of neat microplastic particles and tablets covering a variety of commonly encountered microplastic polymers. These reference materials are rigorously characterized and validated to ensure their authenticity, size, and composition, providing researchers with the tools they need to confidently interpret their analytical data.

This presentation will showcase Chiron's commitment to advancing environmental monitoring and research through the development of high-quality microplastics reference materials. We will discuss the production process, characterization methods, and validation procedures for our reference materials, highlighting their importance in ensuring reliable and comparable microplastic measurements.

### **3.14.P-Mo220 Deposition of Atmospheric Microplastics in an Urban Environment**

*Mine Sağdıç Ulusoy and Eftade Emine Gaga, Environmental Engineering, Eskisehir Technical University, Turkey*

Atmospheric scavenging is known to play an important role in the transport and fate of atmospheric pollutants. Studies have shown that atmospheric microplastics are effectively washed out of the atmosphere by wet deposition. However, dry deposition of microplastics, which is another effective mechanism for the transport of atmospheric MPs, is much less common in the literature. Deposition samples were collected from the urban catchment of Eskişehir, Turkey, using a wet-only sampler. A certain amount of the collected samples was taken for analysis. Centrifugation and oxidative extraction methods were used to remove organic materials in the pre-treatment of the samples. Samples filtered through the polycarbonate filter after pre-treatment were analyzed using a stereomicroscope. MPs were identified and sorted using ImageJ software based on their quantity, size, shape, and color. In preliminary studies, the average dry deposition flux according to the stereomicroscope results was 6310  $\text{MP/m}^2/\text{d}$  for all suspected particles and 995  $\text{MP/m}^2/\text{d}$  for fiber particles. It was observed that the amount of MPs varied between the samples. The subsequent phases of the study will involve the chemical characterization of the MPs in deposition samples that have been visually analyzed. The characterization of the MPs will be used to investigate the scavenging of the MPs by wet and dry deposition.

Keywords: *Atmospheric Microplastics, Dry Deposition, Scavenging, Precipitation, Urban*

### **3.14.P-Mo221 Atmospheric Microplastics in Two Norwegian Cities, Composition and Temporal Trends**

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Small microplastic (MP) particles can be subject to atmospheric transportation or wash out of the atmosphere during precipitation. A growing amount of evidence suggests that MPs are ubiquitous in the air as they are in other environmental compartments, such as the marine environment. However, little is known on local sources and temporal trends.

Here, we present data on the occurrence and composition of atmospheric microplastics in two Norwegian cities, Oslo and Tromsø. Samples were collected in summer/autumn 2023 and include active air samples as well as passive (deposition) samples, with each sampling period covering 14 days. Samples were analyzed for 10 polymers using pyrolysis-gas chromatography–mass spectrometry (Py-GC/MS). Styrene-butadiene rubber (SBR) was the dominant polymer in samples from both cities, representing 80-90% by mass of detected polymers in all samples. This suggests that car traffic is the predominant

source of airborne microplastic contamination in urban areas, independent of the longitude of the study area. The population size of Tromsø is roughly 10 times less than the population size of Oslo, but SBR concentrations were similar or only slightly lower in samples from Tromsø. Possible explanations might be bad road conditions in Tromsø (as typical for cold climate regions) leading to a higher production of tire and road wear particles (TRWP) or a higher availability and use of public transportation in the capital.

### **3.14.P-Mo222 Investigating the Case of Microfibre Pollution in Air: Which Fibres are Guilty?**

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The increasing demand for fast fashion, characterised by rapid manufacturing cycles and disposability, has ushered in a culture in which garment care has become a significant determinant of the industry's environmental footprint. Emissions from drying processes, which release microfibrils (MFs) into the air, have consequences for both indoor and outdoor environments, altering air quality indices and impacting human health. In contrast to the extensively researched field of washing machines, there is paucity of study in cloth dryers' impact. This study therefore aimed at assessing the effectiveness of MF capturing devices built internally within tumble dryers or added during drying process and the role of laundered garments in the prevalence of MF in air when more than one garment type is tumble dried together.

10 garments made from 100% cotton and 10 garments made from 100% polyester were all washed and subsequently dried together in a tumble dryer. The efficacy of lint filters (components of tumble dryers) of varying pore sizes and dryer sheets (external MF capturing device) was determined. In all the experiments, cotton MFs were released significantly more than polyester MFs.

This study evidences the contribution of vented tumble dryers to airborne MF pollution whilst highlighting that a garment's construction is a major determinant to the release of MFs available for polluting the air. Therefore, discussions around airborne MF pollution ought not be centred on synthetic fibres alone as any garment has the potential to contribute to this form of pollution which can be exacerbated by the way it is constructed. Recommendations and new developments in the field based on an ongoing study through the IMPACT+ Network would be presented at the conference.

### **3.14.P-Mo223 Invisible Footprint of Climbing Shoes: Data From Indoor Facilities Reveal Unprecedented Exposure to Rubber Additives**

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There is increasing research focused on rubber-derived chemicals (RDCs), predominantly originating from tire and road wear particles. Other consumer products also contain RDCs, but the overall human exposure to these compounds remains unknown. The soles of climbing shoes are highly engineered for specific properties, such as softness, flexibility, stiffness, and durability. Therefore, climbing shoes could be a contributor to human indoor exposure to RDCs, including potentially harmful substances such as benzothiazoles, p-phenylenediamines (PPDs), and PPD-quinones. In 2018, an estimated 1.5% of UK population, and about 4.4% of the US population visited indoor climbing halls. Therefore, indoor climbing halls might be a relevant but previously overlooked micro-environment where a noteworthy portion of the population, including employees, is exposed to inhalable rubber particles and the organic RDCs they contain. We show that shoes samples (n=30) contain high but variable concentrations of RDCs (mean 711 µg/g). In indoor climbing halls, abrasion particles from these shoes can be suspended in the air. Dust (n = 5) and air samples (n = 2) were collected in two climbing halls and particulate matter in the inhalable and respirable fractions were analyzed for 15 RDCs. Mean PM concentrations were 1295 µg/m<sup>3</sup> in the inhalable and 970 µg/m<sup>3</sup> in the respirable fractions, which exceed WHO guidelines for indoor PM10 concentrations of 50 µg/m<sup>3</sup>. Concentrations in dust (16 to 43 µg/g) and particulate matter (23 to 35 ng/m<sup>3</sup>) exceed those reported from other environments. For most RDCs, estimated daily intake via inhalation (EDI<sub>inh</sub>) for adults visiting or working in these facilities exceeds the EDI from other sources. This highlights the potential concerns with using large amounts of rubber additives in consumer products. RDCs profiles in shoe samples differed from those in dust and particulate matter, indicating that RDCs are chemically transformed in airborne rubber particles, which we confirmed by performing lab-controlled oxidation experiments. This finding has broader implications as similar transformations are likely to occur in airborne tire wear particles. Future research should address the leaching and bioavailability of RDCs within the human body as well as the toxicological risk that RDCs pose in the respiratory and gastrointestinal tracts.

### **3.14.P-Mo224 Occurrence and backtracking of microplastics in Northern Atlantic Air**

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By now microplastic (MP) pollution is shown to be omnipresent and background knowledge on their dispersion and enrichment behavior are of great interest. Meanwhile, the database for many environmental compartments is gradually becoming solid, except for remote areas such as the oceans when air masses become into focus. While sampling on land is comparatively easy to realize, areas, especially over the oceans, are difficult to access. To date, little is known about the pollution of the marine atmosphere with microplastics and tire wear particles. The very few studies available and they are almost exclusively particle-based. Mass-based ones are extremely rare and incomplete. The presented study is embedded in the JPI-Oceans FACTS project dealing with occurrence and transport of MP up to northern waters. During a research cruise in 2021 seven transects along the Norwegian coast up to Bear Island were actively sampled on the observation deck during steaming to prevent any ship based secondary contamination. The performance of two different sampling devices was evaluated. MP analysis and mass quantification was conducted using a by now established Py-GC/MS method referring to defined base polymer clusters (C-) related to specific base polymer building blocks. With careful reference to available field and laboratory blank values, MP was detected even in remote Arctic areas with concentrations up to 37.5 ng MP m<sup>-3</sup> air and a clear predominance of the PET cluster. In addition, car tire tread, and clusters of PS, PP, and PUR were detected more often. Using two independent "backtracking" models (HYSPLIT and FLEXPART) an attempt was made to reconstruct the origin of the air masses and to gain information about the origin of the measured MP from it. In addition to the more obvious aeolian transport of MP particles, the re-emission of MP from the ocean into the overlying air layers appears to be a relevant source in this context. Likewise, in particular the long-range transport of C-PET particles appears to be substantial. The range of detectable polymers, but also the risk of sample contamination, was closely linked to the particular sampling method used [1].

[1] Goßmann I., Herzke D., Held A., Schulz J., Nikiforov V., Georgi C., Evangelidou N., Eckhardt S., Gerdt G., Wurl O., Scholz-Böttcher B.M. 2023. Nature Communications (2023)14:3707, doi10.1038/s41467-023-39340-5.

### 3.14.P-Mo225 Extraction of Aged Bio-Microplastics from Compost Matrices: Method Development

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Biodegradable plastics have been seen as a promising alternative to conventional plastics, offering the potential to mitigate the environmental burdens associated with the latter at multiple stages of their lifecycle. Biodegradable plastics are often designed to be degradable in industrial waste management settings. However, current test methods to assess their degradability (e.g., during industrial composting) do not consider the potential for microplastic production if complete degradation is not reached. While extensive research has explored the presence and impact of microplastics from conventional plastics in various environments, a significant challenge lies in extracting and assessing microplastics originating from biodegradable plastics (bio-microplastics). Current methods for microplastic extraction employ chemicals that can be destructive to bio-microplastics, especially if they are aged. This challenge necessitates the development of specialized methodologies tailored to extract and quantify aged bio-microplastics. Detecting and analyzing bio-microplastics is crucial to understanding further biodegradable plastics' environmental fate and potential negative impacts. This study introduces the development of a novel method for extracting aged bio-microplastics from compost matrices. Utilizing the oleophilic nature of plastics, this method employs oil to extract microplastics. During the method development, varying parameters, including sample amount, amount of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) used for the pretreatment, and oil type, were explored alongside pretreatment without H<sub>2</sub>O<sub>2</sub>. The quantification and simultaneous chemical characterization of microplastics were performed via  $\mu$ -FTIR. The study aimed to provide methodological guidance on future procedures for isolating and analyzing bio-microplastics from complex matrices, such as compost. By addressing the limitations of existing microplastic extraction procedures, the study aims to shed light on the often-overlooked aspect of biodegradable plastic degradation and its potential contribution to microplastic pollution.

### 3.14.P-Mo226 What is in specks of highway road dust? Quantification and Chemical Characterization of Small microplastics (<100 $\mu$ m) and Plastic additives

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Highway road dust (HWRD) continuously accumulates on highways due to the traffic of vehicles; it is a composite miscellany of particles, and it is considered one of the primary sources of microplastics (MPs) in the atmosphere. The wear of tires, bitumen, road marking paints used in road pavement, brake wear, vehicle emissions, soil from construction sites, atmospheric deposition, plastic or other materials debris, and road pavement made of recycled plastics are generally deposited in the HWRD.

In this study, a simultaneous quantification and chemical characterization of additives, plasticizers, natural and non-plastic synthetic fibers (APFs), and small microplastics (SMPs, <100  $\mu$ m) in HWRD will be performed. The sampling procedure was optimized, as well as pretreatment (extraction, purification, and filtration) and analysis via Micro-FTIR. HWRD samples were collected during dry periods (at least 2 weeks after a rainfall event) from winter 2021 to winter 2022 from a trafficked highway in Italy.

A detailed protocol was designed to minimize potential plastic contamination of samples during sampling, transport of samples, pretreatment, and analysis. The pre-treatment procedure (oleoextraction, purification, and filtration) was optimized according to the one previously developed. The filters were then analyzed via Micro-FTIR, where identification and quantification via microscopic counting were performed simultaneously.

Among the polymers characterized and quantified, vinyl ester and polytetrafluoroethylene were predominant. Lubricants and plasticizers are the two most abundant categories, followed by vulcanizing agents, accelerators, and pre-vulcanizing retarders derived mainly from tires. A potential relation between APFs and SMPs in the different seasons was observed, as their concentration was lower in summer for both and higher in winter 2022.

Data from this study will be relevant in assessing the load of SMPs and APFs from highways, which is urgently necessary for a more accurate inclusion in emission inventories, receptor modeling, and health protection programs by policymakers, especially in air and water pollution policies, to prevent risks for human health.

### **3.14.P-Mo227 Tire Particles and Microplastics in Urban Road Dust: A Double Shot Pyrolysis-Gas Chromatography-Mass Spectrometry Analysis for Identification, Characterization and Quantification**

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Road dust consists of a combination of organic and inorganic particles, among which Microplastics (MPs) were found to contribute up to 0.06% of dry mass within an urban city environment. MPs have been detected in road dust worldwide and their presence has been mainly attributed to the vehicle traffic, resulting in the fragmentation of large pieces of roadside debris, including plastic food containers and packaging as well as asphalt and other construction materials, soils and organics.

The identification of MPs in environmental samples is complex; current analytical methods vary greatly in sampling techniques as well as extraction and analysis protocols. It is believed that the microplastic pollution in road dust has been underestimated, due to inefficient detection methods, especially for tiny MPs. Spectroscopic techniques may be used to identify MPs, such as Raman or Fourier Transform Infrared (FTIR) spectroscopy, nevertheless only chromatographic techniques coupled with pyrolysis-gas chromatography allow the mass quantification of tire particles and MPs independently of their size.

A double shot (thermodesorption and pyrolysis) TD/PY-GC-MS method was applied to urban dust samples to identify and quantify, very selectively, tire particles and MPs, in addition to performing a qualitative analysis of other plastic additives and organic contaminants present in the sample.

The sample is sieved into different fractions, 63, 125, 250, 500, 1000 and 2000  $\mu\text{m}$  and each of these fractions is introduced directly into the pyrolyzer, without any pre-treatment. Several polymer-specific markers are used to confirm the presence of tire particles and MPs, and the different polymers present in each of the fractions are identified and quantified.

Polymers such as PS, PVC, PC, PMMA and tire particles were found in the smaller particle fractions. However, PE and MDI-PUR were found only in the larger particle fractions. Furthermore, compounds such as octylphenols, PAHs, benzothiazols, phthalates, alkyl citrates, fatty acids, squalene or levoglucosan were identified thanks to the thermodesorption method.

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### **3.14.P-Mo228 Fabrication and Analysis of Microplastics Reflecting Weathering Characteristics**

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Microplastics, due to their extremely small size and long-lasting presence in the environment, are globally recognized as a significant concern with potentially harmful impacts on ecosystems and human health. Despite widespread research on the human and environmental toxicity of microplastics, much of the currently utilized microplastics in studies often fail to accurately reflect the characteristics and forms of microplastics detected in the environment. Microplastics exist in altered states, with changes in shape and surface properties, resulting from physicochemical weathering. The majority of research relies on commercially available, limited-sized spherical polymer particles. Therefore, there is a need for studies focused on weathered microplastics that actively reflect the features of microplastics prevalent in actual marine environmental pollution. In this study, various polymeric materials were milled, powdered, and fractionated to produce samples of different sizes and shapes for toxicity assessment. Due to the inherent ductility of polymers and melting issues during milling, a cryogenic grinding method was employed for sample preparation. Electron beam irradiation was conducted as a preprocessing step to obtain particle sizes in the range of several micrometers, confirming the effectiveness of electron beam irradiation in particle size reduction. Through cryogenic milling and precise classification processes, uniform particle sizes ranging from less than 20

µm to more than 150 µm on average were produced. The developed microplastic model samples are being utilized in assessing environmental behavior and toxicological impacts. With the capability to produce samples reflecting actual weathering characteristics, this advancement is expected to significantly enhance the scientific reliability of microplastics research.

### 3.15.A Measuring and Modelling the Environmental Fate and Exposure of Pesticides

#### 3.15.A.T-01 Weight-of-evidence approach for correlation of degradation and/or sorption parameters with soil pH

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The FOCUS kinetics guidance (2014) provides a harmonised approach to consider degradation of parent pesticide compounds and breakdown products to determine key regulatory endpoints (DT<sub>50</sub>, DT<sub>90</sub> and formation fractions) to support pesticide risk and hazard assessments. OECD guideline 106 provides guidance on the conduct and interpretation of adsorption/desorption studies also used to determine key regulatory endpoints (K<sub>loc</sub>, 1/n). However, when considering potential correlation of degradation or sorption parameters with soil pH, there is no specific EU guidance available on how to investigate and demonstrate such a relationship, as noted in the EFSA scientific report on surface water repair (EFSA, 2020).

Through extensive experience of evaluating many active substance packages, HSE's Chemicals Regulation Division (CRD) have developed a weight-of-evidence approach to help concluding on correlation of degradation and/or sorption parameters with soil pH. The approach recommended here is split into two steps to ensure that a consistent and robust conclusion is reached. First, a review of all relevant information should be undertaken to establish if there are good scientific or mechanistic reasons to suspect that a correlation exists. Second, the significance of the correlation is investigated via adequate statistical tests. When supported by the outcome of steps 1 and 2, the exposure assessment should be performed accounting for any significant correlation.

Due to the complexity of interactions in soils and the typically limited nature of regulatory datasets it is possible that a number of different and potentially contradictory outcomes will result from Steps 1 and 2. Where a high degree of uncertainty exists, consideration should be given to ensuring that a conservative assessment is conducted at the first tier based on the evidence supporting a clear mechanistic basis for a relationship to be expected.

#### 3.15.A.T-02 Uncovering the Spray Drift in Unscrewed Aircraft Spray Systems Through Intensive Field Studies

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Uncrewed Aerial Spray Systems (UASs) have emerged as a promising technique for the application of crop protection products, offering potential benefits for agricultural efficiency and sustainability. However, off-field deposition, known as spray drift, poses risks to crops, the environment, and humans. This study presents the results of 114 UASs drift trials conducted in China in 2022. The analysis focuses on quantifying the spray drift of UASs and investigating the impact of environmental factors, including wind conditions, air temperature, relative humidity, as well as UAS flying altitude and speed. The study found that UASs drift magnitude falls within a similar range to the one of air blast application measured for orchards in the EU. Under the experimental setups, the study demonstrates a higher drift when the UASs was flying at 5m/s speed and at 5m altitude, compared with 3m/s speed and 3m altitude. Results of an ANOVA test indicate that environmental variables have a more significant impact on drift values than spray system parameters adopted by the experimental setups. The study highlights the importance of considering multiple factors when analysing drift behaviour and provides valuable insights for risk assessment and responsible use of UASs applications of crop protection products in agriculture.

#### 3.15.A.T-03 Appropriateness of the Soil Organic Matter Map contained within PERSAM: Exploring Options in a Dynamic and Evolving GIS Data Landscape

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The EU soil risk assessment for the approval of plant protection products (PPPs) is under revision (EFSA, 2017). European-wide GIS data layers and analytical equations implemented through the PERSAM tool are proposed to calculate predicted environmental concentrations in soil (PECsoil). PECsoil values at all tiers are significantly impacted by soil Organic Matter (OM) content, as soil Bulk Density (BD) is derived from OM content with an empirical equation, and thereby the OM GIS layer is a critical driving factor in the proposed modelling scheme. European-wide soil OM GIS layers generated using various approaches are increasingly available. However, all such layers include inaccuracies and biases when compared to real-world measurements. For example, the EFSA OM map available with the PERSAM software, exhibits significant biases in arable land areas. Nonetheless, an EU soil risk assessment based on flawed data may result in safe PPPs being inappropriately removed from sale. Thus, to evaluate the representativeness and PECsoil impact, a systematic analysis of available European soil OM data layers (e.g. EFSA 2012 OM map, de Sousa et al. 2022) has been performed, comparing them with measurements of real soil OM on arable land in Europe, obtained from LUCAS (2018) database. Arable land was defined as the spatial union

of three classes (i.e. “Annual crops”, “Permanent crops” and “Rice”) of the CORINE Land Cover (CLC) 2012 layer. The comparison with LUCAS 2018 OM measurements confirmed significant inaccuracies within the EFSA 2012 OM map used in PERSAM. Analysis of the EFSA 2012 dataset also demonstrates agronomically unrealistic soil conditions with OM content exceeding 10% in most cases, with maximum values of 20% in the Northern Registration Zone. The analysis also suggests EFSA 2012 may be in general the least accurate European OM dataset among those currently available. The authors hope this work may favour a scientific and regulatory discussion around the digital mapping of OM in Europe, to implement a robust GIS layer of OM based on the best science available, using updated sources and comprehensive datasets. To conform with the EC “FAIR” commitment to guarantee findable, accessible, interoperable and reusable data, a traceable and well-defined procedure should be also defined to periodically update and check the spatial layers which form part of the EU PPP registration process.

### **3.15.A.T-04 Harmonised Framework for the SETAC Spatially Distributed Leaching Modelling of Pesticides Initiative: 2024 update**

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Spatially distributed leaching modelling (SDLM) of pesticides is a methodology to estimate the leaching potential over a large spatial extent such as national or European scale. It is described in the FOCUS groundwater report and foreseen to be used as higher tier leaching risk assessment as well as supporting groundwater monitoring studies. At the SETAC Europe 2020 meeting, the initiative was officially formalised as a SETAC working group, consisting of a triad of members from regulatory agencies, academia, and the private sector. As the SDLM team continues to work, this presentation provides an update to interested parties.

The group has now developed a first version of the framework. The process-based leaching models PEARL and PELMO will form the core of the framework. The model will run for approximately 10,000 scenarios, which are unique combinations of land-cover, climate and soil data. The scenarios were created from the geodata using a *k*-means clustering procedure, which aims to minimise within-cluster variances. The group has selected datasets that cover the entire EU and UK. The framework will use soil data from the SoilGrids database. Because the soil organic matter content depends on land-use, a map specific for arable soils was developed. For this, machine learning algorithms were applied using the available data in the SoilGrids database. The group paid a lot of attention to the harmonisation of pedotransfer functions. For this, available pedotransfer functions for soil bulk density and soil hydraulic properties were reviewed.

The framework will be tested using several test substances. Specific attention will be given to the plausibility of the predicted leaching maps. Furthermore, the 90<sup>th</sup> overall percentile leaching concentration for the nine FOCUS zones will be calculated and compared with results from the nine FOCUS scenarios that are used in Tier-1.

The work so far demonstrates that the development of a framework involves expert judgements that need to be thoroughly checked and documented. A plausibility check and a comparison of different frameworks is therefore part of this project. However, in the long run, a monitoring network consisting of multiple field sites would be helpful to further gain confidence in the modelling tools.

### **3.15.A.T-05 Preparing to revise the Guidance Document on risk assessment for non-target arthropods to pesticides: off-field exposure characterisation**

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The EU legislative framework on Plant Protection Products (PPPs) underscores the significance of conducting thorough risk assessments for active substances to protect Non-Target Terrestrial Organisms (NTTOs). The focus has recently been on revising guidance documents for bees, birds and mammals. Now, there is a high priority emphasis on Non-Target Arthropods (NTAs). In this remit, EFSA set up a Framework Partnership Agreement with Wageningen University and Research with the aim to contribute to the development of concepts and methods for the environmental exposure assessment of NTTOs to pesticides. The project is structured to address key steps in this assessment. One pivotal aspect is the definition of the strips/areas surrounding pesticide-treated fields where crops are grown. It is recommended to focus on three defined exposure

strips: the in-crop area, the in-field off-crop strip, and the off-field strip. These serve as the foundation for Specific Protection Goals (SPGs), which are critical in determining which ecosystem services need protection. Another crucial step involves outlining environmental exposure routes for off-crop NTTOs, with special focus to spray drift depositions. The project also proposes a structured approach to develop higher tier exposure scenarios for NTAs. The outcomes of the project stress the importance of clear definitions and identify data gaps, such as the lack of spray drift deposition data on in-crop or off-crop plants as 3-dimensional (3D) structures. The valuable role of this work is highlighted in jump-starting scientific initiatives for the development of exposure scenarios for NTAs and for the plausibility check exercises conducted with existing spray drift models to verify if they can be effectively used across EU regions. In conclusion, the collaborative efforts aim to establish a comprehensive and harmonised framework for assessing the environmental risk of pesticides on NTTOs, providing a solid foundation for future regulatory guidance.

### **3.15.B Measuring and Modelling the Environmental Fate and Exposure of Pesticides**

#### **3.15.B.T-01 Improving the procedure for fitting degradation rates in water sediment studies**

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In aquatic risk assessment studies it is typical to determine total system degradation and/or dissipation half lives from water-sediment experiments rather than individual degradation rates of the water and sediment phases. The disadvantage of this is that the use of total system half lives or dissipation half lives may mischaracterise the environmental fate processes in aquatic systems.

The reason that compartment-specific degradation rates are often not used in aquatic risk assessments is that it is difficult to obtain statistically robust estimates of these rates in the experimental system (e.g. excluding the initial mass,  $M_0$ , 4 rate parameters need to be fitted from approximately 8 - 12 time points), whereas it is easier to obtain total system degradation or dissipation half lives (e.g. excluding  $M_0$ , 1 rate parameter is fit for in single first order kinetics).

In this work we propose to use the sediment water sorption relation ( $K_d$ ) to reduce the number of fitting parameters and thus increase the likelihood of statistically robust fits. Case studies will be presented to show the difference in fitting for 4 rate parameters versus 3 rate parameters. Since the number of time points in water sediment studies is typically around 8 to 12 (i.e. not very large), there is a large gain in certainty from only fitting 3 rate parameters rather than 4. However, whilst fitting for 3 parameters improves the robustness of results, robust fits are not always guaranteed. Therefore, the uncertainty of fitted parameters across theoretical parameter space was also investigated. It was found that robustness of back transfer ( $\alpha_s$ ) is high except for when both sorption and  $\alpha_s$  are high, and certainty of degradation in water ( $k_w$ ) is highest when  $k_w$  is large, particularly when sorption and  $\alpha_s$  are low. Conversely, degradation in sediment ( $k_s$ ) is most accurate when  $k_s$  and sorption are high. To improve the procedure further it is possible to use results from other experimental data (e.g. a concomitant OECD 309 test).

In summary, current regulatory methods often only yield total system degradation rates and dissipation rates of the specific compartments. This may lead to an overly conservative risk assessment, especially if behaviours over long time frames are considered (e.g. FOCUS SW repair). Conversely the simplified approach proposed here allows the decoupling of degradation and sorption and hence more accurate parameterisation of surface water models.

#### **3.15.B.T-02 Comparison of simulated and observed downwind deposits of spray drift in arable crops across Europe: a test case using the IDEFICS model**

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EU Regulation 1107/2009 requires the assessment of pesticide exposure risk for several groups of non-target organisms, such as Non Target Arthropods. Spray drift deposition is a major exposure route, that has not yet been estimated reliably at an European level, while accounting for the wide variety of factors and conditions across Europe. Typically, this variety is caused by differences in crops, crop height, agronomic practices and agro-environmental conditions.

Mechanistic spray drift models can be helpful to deal with this variety by appropriately parameterizing the above-mentioned factors and conditions. By demonstrating that mechanistic models can predict spray deposits for a range of conditions sufficiently well, modelled depositions may improve the current exposure assessment within the regulatory risk assessment at EU and Member State level. A few mechanistic spray drift models exist in Europe that take account of such factors and conditions. In principle, these models can be tested to see how they perform in a relatively wide range of situations compared to experimental field trials. In this study the Dutch IDEFICS model (Holterman et al, 1997) was selected to carry out this comparative test. IDEFICS computes downwind deposits of spray drift for arable crops with conventional boom sprayers. Computed spray drift deposits are a function of crop type and height, sprayer settings and meteorological conditions. However, this model has only been tested and validated for Dutch situations. A set of experiments was selected from the EU-wide database of SETAC-DRAW and a few other sources, complying to predetermined criteria, to test the wider validity of

IDEFICS estimates of spray drift deposition. In total, five datasets were selected covering short and tall crops in the three different EU regulatory zones (North, Central, South), kindly provided by various research groups in Europe. Field trials generally consist of several replicates, each experiencing slightly different e.g. meteorological conditions, such as wind velocities, therefore each replicate needs to be simulated separately. The comparison shows that mechanistic spray drift models can be useful to account for environmental and conditional differences across Europe. However, experimental variability still remains an important issue that has to be dealt with adequately.

### **3.15.B.T-03 Estimating high resolution exposure at landscape-level – on the development, evaluation, and application of the droplet and atmospheric dispersion (DAD) drift model**

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Information on the fate and transport of pesticides is of great interest for pesticide registration, product stewardship, authorities, and other stakeholders. Major transport pathways include surface and subsurface runoff, leaching, subsurface drainage, and spray drift. Modeling of pesticide fate and transport within watersheds, where pesticide transport is controlled by the dominant hydrological processes, has been increasingly applied at the plot scale and the landscape scale. However, due to its complexity, the process of spray drift is largely neglected or oversimplified in landscape-level modeling approaches.

With the Droplet and Atmospheric Dispersion (DAD) drift model, we have developed a computationally efficient modeling approach, capable of accounting for the physical basis of spray drift, the relative location of application areas to non-target areas, and local weather conditions at the landscape scale. The DAD drift model is a combination of a mechanistic droplet model, a micrometeorological model, and a three-dimensional Gaussian puff model and was developed for ground application.

The model was successfully evaluated against two field-scale spray drift studies for ground application covering a wide range of drift potentials. In a sensitivity analysis, DAD drift showed coherent and comprehensible behavior for variations of important input parameters (e.g., different nozzle types, wind speed). In a virtual experiment, the model was compared with different drift prediction approaches using simplistic algorithms based on drift curves. Results indicate that predictions of DAD drift are within the range of other landscape-level approaches. However, for individual elements in complex landscapes, significant differences in drift predictions up to several orders of magnitude can be found. Furthermore, DAD drift was applied in a meta-modeling approach using generalized linear models (GLM). The nozzle type in terms of the drop size distribution, closely followed by the distance between application and non-target area, were found to be the two most influential drivers of spray drift at the landscape scale.

To further improve the understanding of pesticide transport pathways at the landscape scale, work on a high-resolution SWAT+ model of an agriculturally dominated catchment in Germany in combination with the DAD drift model is ongoing.

### **3.15.B.T-04 Model-Based analysis of pesticide transport pathways in a drained agricultural farm, northern Germany**

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Pesticides are currently detectable in all types of water bodies and pose a serious threat to the environment. Waters in the northern German lowlands are particularly at risk, as subsurface drainage of cropland quickly transports pesticides, including persistent transformation products (TPs), to surface waters. The eco-hydrological model SWAT+ is a useful tool that links hydrological processes with agricultural practices and the degradation and transport of pesticides. In this study, the focus is on the simulation of three herbicides (pendimethalin, diflufenican, and flufenacet) with different environmental behaviours, as well as the simulation of two TPs of flufenacet, flufenacet-oxalic acid (FOA) and flufenacet-sulfonic acid (FESA). SWAT+ is applied on a 100-hectare farm with an extensive drainage network of 6.3 kilometres. The farm is managed using conventional agricultural methods.

SWAT+ models the discharge of the area with very good agreement during calibration and validation. For pesticides, the model performance for the highly mobile TPs and the moderately mobile flufenacet is better than for the non-mobile diflufenican and pendimethalin. The tile drain transport of moderately to very mobile substances plays a significant role in the total losses. The contribution of tile drain transport increases with increasing time after the application and can dominate in phases of peak losses. No transport via tile drains can be modelled for the non-mobile pendimethalin, as particle-bound pesticide transport via tile drains is not simulated in the model.

We conclude that SWAT+ is capable of modelling the transport and degradation of moderately mobile pesticides and very mobile TPs and can be used for the evaluation of transport pathways. However, the model has limitations when considering the underground transport of non-mobile substances, which can lead to an underestimation of the total losses.

### **3.15.B.T-05 Moving towards field specific risk assessment: A drainflow risk assessment case study in the UK.**

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The registration of plant protection products in Great Britain includes an assessment of risk to surface water via agricultural drains using the MACRO preferential flow model. The drainflow risk assessment considers the number of years in which the regulatory acceptable concentration (RAC) is exceeded for 20 pedo-climatic scenarios. Depending on the species driving the RAC up to 3 (aquatic invertebrates and fish) or 18 (aquatic plants and algae) out of the 30 years simulated may exceed the RAC in any given pedo-climatic combination. As such, even if a small number of pedo-climatic combinations fail the risk assessment no product registration is possible even though the simulations indicate that there are many pedo-climatic combinations which demonstrate safe usage along with all undrained soils.

This poster outlines a spatially distributed drainflow modelling (SDDM) approach that allows (a) vulnerability maps that identify areas that may pose a risk to edge of field waterbodies to be established and (b) decision support tools to be developed that facilitate farmer compliance with spatially varying label restrictions that preclude application to specific drained soil series. The Macro model based SDDM defines a broader suite of drained soils classes as well as simulating exposure for all MARS weather grid squares in GB thereby capturing a wider range of pedo-climatic combinations associated with arable land than the standard scenario-based approach. By aligning the risk assessment of these SDDM combinations with the current regulatory approach 3 broad classes of vulnerability are defined: (1) areas where no drained soil classes are predicted to exceed the RAC; (2) areas where all drained soils exceed the RAC and (3) those where some intermediate risk is identified requiring further investigation. Such an approach is closely aligned with the existing regulatory modelling and decision-making processes to facilitate regulatory adoption. It is also aligned with the increasing prevalence of precision agriculture, digital pesticide labels and field specific mitigations to achieve required product label mitigation levels. Moving to a field specific risk assessment approach would allow GB growers access to a more diverse suite of plant protection products whilst maintaining protection of the environment.

### **3.15.C Measuring and Modelling the Environmental Fate and Exposure of Pesticides**

#### **3.15.C.T-01 Occurrence and Bioaccessibility of Pesticides in Agricultural Plastics**

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Intensive agriculture employs large amounts of plastics (i.e., greenhouse and mulching films, anti-grass covers, plant protection tubes, irrigation pipes and tying systems) to increase the productivity of crops. The above materials are exposed to pesticides sprayed on crops. Depending on the features of the employed compounds, the type of polymer and its weathering grade, these compounds might be sorbed and/or accumulated in plastics. Physical degradation of agricultural plastics leads to smaller size pieces, difficult to recover from fields, and susceptible to migrate to other environmental compartments.

Recycling of agricultural plastics has become a common practice to reduce the environmental impact of end-of-life materials, limiting the generation of microplastics in the terrestrial environment. Considering sell figures, low density polyethylene, employed in greenhouse and mulching, is one the most important of the recycled agricultural plastics. Pesticides sorbed in the aged material might be transfer to pellets produced in the recycling process. Thereafter, they might even reach the second-hand items prepared using this recycled raw material.

Meso- and microplastics generated from agriculture use materials are practically impossible to collected from crop soils. Moreover, they are susceptible of ingestion by wild animals and life-stock feeding on non-commercial rest of crops. On one hand, a fraction of pesticides associated to ingested plastics might be accessible in the digestive tract of animals. On the other hand, plastics and associated pesticides are transported and excreted at significant distances from the generation site.

The aims of this presentation were (1) to characterize the composition of plastic debris collected from agricultural plots, (2) to assess the presence of pesticide residues in these materials as well as in new plastic items, suspected to be prepared from recycled plastics, and (3) to evaluate the bioaccessibility of pesticides sorbed in agricultural plastics during simulated digestion experiments. The characterization of plastic debris was made by infrared spectroscopy. Determination of pesticide residues was carried out by liquid chromatography tandem mass spectrometry (LC-MS/MS), after solid-liquid extraction. Finally, the stability of sorbed compounds and their bioaccessibility during simulated digestion of aged materials were investigated under standardized conditions, using aged polymers from agricultural fields.

#### **3.15.C.T-02 Testing the PEC-CKB Model with Data from a Monitoring Study in Small Freshwater Streams in Germany**

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Predicted environmental concentrations (PEC) result from mathematical fate models and are used as a quantitative indicator for the expected occurrence of chemicals in the environment. Recently, the PEC-CKB model (Boström et al., 2019) was suggested as an additional possible tool for prospective exposure assessment, and stands out for its simplicity. The regression

model equation includes factors such as application dose (D), transport loss (M) and substance-specific sorption behaviour ( $F_w$ ) as well as a dilution factor, and results in a simplified equation  $PEC=0.5*D*F_w$ . With that, good results have been obtained by comparing model-generated PEC with Swedish monitoring data.

This study, which is part of the PARC (*European Partnership for the Assessment of Risks from Chemicals*) project, aims to test the PEC-CKB model with German monitoring data obtained from the Kleingewässermonitoring (KgM) (Liess et al., 2021). The monitoring data comprised of 124 sites and 108 substances were measured between 2018 and 2019. Model results using original parameters significantly overestimated measured environmental concentrations (MEC) in Germany, in contrast to prior research in Sweden. This has prompted an investigation into regional and seasonal variations in environmental conditions. By using the average application doses given for Germany, already more accurate model results could be obtained. Additionally, we are examining the land use in various catchments by using geographical information systems (GIS). From the expected results we aim to derive site-specific parameter values to enhance the model's performance in Germany by including country-specific factors in the model equation. Finally, the PEC-CKB model, although conservative, seems suitable for lower-tier analyses. It appears advisable to match the intended objectives, taking into account any variances in accuracy and protection levels when choosing models.

### **3.15.C.T-03 > Sixty-Year Historical Records- Fate and Persistence of Plant Protection Products in Aquatic Systems**

*Aurea C. Hernández, Emmanuel Schaad, Nichole Fahrni, Olivia Wagner, Ann-Kathrin Stalder and Martin Grosjean, University of Bern, Switzerland*

Plant protection products (PPPs), applied to agricultural fields in the range of hundreds of thousands of tons per year in Europe, pose rapidly growing risks to soils, atmospheric and aquatic environments, ecosystems in general, and human health. The pathways of PPPs through the environment are highly complex and need to be better understood. Although many studies have analyzed the losses of diffuse PPPs from agricultural fields to surface water (e.g., rivers and creeks), few attempts have been made to comprehensively understand the transport and deposition of current-use PPPs in freshwater lake systems. In addition, the ability to assess PPP loads in the dissolved and particulate phase is a key issue that has not yet been adequately addressed by models due to limited or non-existent data to evaluate deposition and re-emission processes in sediments.

Our work uses a novel multiproxy workflow to assess > 60 PPPs in sediment samples from different Swiss lakes under heavy agricultural pressure. The results show the appearance of PPPs for the first time in the early 1960s, with an overall detection of more than 30 PPPs per lake. Paleolimnological data (e.g., chronology, hyperspectral imaging of sedimentary green pigments, and semi-quantitative elemental composition ( $\mu$ XRF scans)) combined with chemical analysis (LC-MS/MS and HRMS) shows that once PPPs are embedded in sediments, they are often quite stable and PPP trends over time (since the 1960s) are not related to land surface processes such as soil erosion or lake biogeochemistry, but are mainly attributed to PPP application (inferred from sales) or regulatory measures (bans). In addition, our work shows that PPPs are not equally distributed in sediments across space, but they can be readily scavenged and removed from the water body. In addition, our work shows that physicochemical properties to predict the accumulation of pesticides in sediment show limitations and often fail to predict their behaviour.

Lake systems like those abundant in Europe are often used as freshwater supplies. Therefore, our findings likely represent many agriculturally influenced lakes in Europe.

### **3.15.C.T-04 Contaminated Landscapes – Current Use Pesticides Residues in Soil and Vegetation along Altitudinal Gradients in a European Alpine Valley**

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Pesticides applied in crops are transferred to non-target areas, exposing biota. We examined current used pesticide (CUP) distribution in terrestrial habitats by sampling soil and vegetation as important exposure matrices for insects in an Alpine valley. Sampling was conducted along eleven transects extending from the valley floor at 517 m a.s.l. to mountain peaks at 2.318 m a.s.l. in the Vinschgau valley (South Tyrol, Italy), Europe's largest apple growing area. We used a recently developed extraction method followed by a high-performance liquid chromatography coupled with an electrospray ionization tandem mass spectrometry system (HPLC-ESI-MS/MS) for 97 target CUPs. A total of 27 CUPs (10 insecticides, 11 fungicides, and 6 herbicides) were detected, originating mostly from apple orchards in the valley. Only one of the 53 vegetation samples was free of CUP residues. CUP numbers and concentrations decreased with altitude and distance to orchards. Valley areas without apple cultivation showed more homogenous contamination along altitudinal gradients, suggesting a valley-wide transport, also into nature conservation areas in high alpine habitats. The insecticide methoxyfenozide was detected in almost half of the vegetation samples, potentially exposing insects orally. Subsequent regression analysis and mapping based on detected residues suggest that CUPs occur everywhere, from the valley floor to mountain tops. This first terrestrial landscape-scale investigation of CUP residues in the European Alps demonstrates widespread multiple residue contamination creating chemical landscapes. Current risk assessment does not consider mixtures of CUPs and measurements in vegetation and soils are scarce. Based on our results, we recommend that the European soil monitoring law must include CUPs and analyse several substances at the same time, as there is currently no data available on realistic exposure of terrestrial ecosystems and the biota inhabiting them.

### 3.15.P Measuring and Modelling the Environmental Fate and Exposure of Pesticides

#### 3.15.P-Mo229 Particulate and Mineral Associated Organic Matter Contribution to Pesticide Sorption in Agricultural Soil

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Intensive agricultural practices heavily rely on land exploitation and high inputs of pesticides to produce crop monocultures. Some applied compounds persist in soil, whereas the more mobile pesticides run off or leach through the soil profile and enter surface and groundwater sources. Soil organic matter content is recognized as one of the main factors determining the mobility and transformation fate of agrochemicals. However, to the authors' knowledge, the role of particulate (POM) and mineral-associated (MAOM) organic matter pools in determining pesticide fate and the implications for soil management practice choice is yet to be elucidated. This research aims to understand how the distribution of organic matter into the physical pools of POM and MAOM influences the sorption of pesticides in soil.

Using wet sieving, we fractionate the soil into the two physical fractions POM (> 53 µm) and MAOM (< 53 µm). After removing organic matter from POM and MAOM fractions, we obtain the rPOM and rMAOM, which serve as control treatments. We perform batch sorption tests to determine the efficiency of 9 pesticide removal from soil solution using separated and treated soil fractions. Our hypotheses are: 1) separated soil fractions with intact organic matter (POM, MAOM) will perform better in pesticide sorption than rPOM and rMAOM; 2) the nature of organic matter determines the extent of pesticide sorption efficiency, and MAOM will be more efficient than POM. The findings of this research can provide new insight into the effect of soil properties in reducing pesticide runoff, leaching, and subsequent environmental contamination and thus have important implications for choosing agricultural land-management practices (tillage, animal manure application, etc.).

#### 3.15.P-Mo230 On the search for alternative herbicides to treat Swiss railway tracks.

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Weeds can have detrimental effects on the railway infrastructure. This mainly through the accumulation of humus, which can increase water retention and may damage both sleepers and rails, as well as the overall stability of the ground. Ultimately, these factors can result in track deformation, which directly influences the speed at which trains are able to travel.

In Switzerland, herbicides are predominantly utilized for weed control, although alternative methods such as mechanical and thermal techniques are also employed. Currently, glyphosate is the only herbicide used to remove weeds from railway tracks, which has proven to be relatively effective. However, uncertainties concerning the renewal of authorisations of the substance in Europe have prompted railway companies to explore alternative herbicides that may replace glyphosate. Due to the inherent permeability of railway tracks and therefore increased net infiltration, it is important to choose herbicides with a low risk of leaching to groundwater. The characteristics of a suitable herbicide include thus a high sorption in the soil and/or rapid degradation (also for metabolites), as well as low application rates.

In order to help decision-makers to make informed decisions, we constructed ten lysimeters with railway materials from three locations, allowing us to study the leaching behaviour of herbicides in close-to-real conditions. Using liquid chromatography–mass spectrometry, we investigate the leaching potential of 12 alternative herbicides and their main metabolites. The selected group of alternative herbicides includes six auxin-mimics, three acetolactate synthase inhibitors, as well as three pigment-synthesis inhibitors.

Half a year after the first application of the substances in May 2023, we observe that leaching is influenced by both the lysimeter soil properties and the herbicides used. While some of the herbicides have already reached their maximum concentrations under all soil conditions, some have not been detected yet. These undetected herbicides may have degraded completely or may still elute later. The project will continue for at least 2 years (from the time of the first application) and will allow us to conclude on the leaching potential of the applied substances and their metabolites under realistic conditions. Moreover, the project will allow a direct comparison of all applied substances under identical weather conditions in 3 different soil types.

#### 3.15.P-Mo231 Concentration dependence of the persistence of the fungicide tebuconazole in fresh water - results of a one-year mesocosm study

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Understanding the consequences of the increasing use of pesticides in agriculture and the associated entry into terrestrial and aquatic ecosystems is essential to efficiently protect the biosphere from collateral damage. Especially the risk assessment of pesticides considering their persistence, which is usually derived from standard degradation tests, may not be safe, since the

half-life of substances can be driven by site-specific conditions, as well as e. g. inhibition of microbial degradation capabilities. Tebuconazole (TBZ), classified as persistent, is a water-insoluble triazole fungicide with inhibitory effects on fungal growth. The use of water-soluble formulations containing TBZ and co-formulants during agricultural activities, led to detectable concentrations in the low  $\mu\text{g/L}$  range in freshwater ecosystems.

In order to investigate the degradation kinetics of TBZ in an aquatic environment, freshwater mesocosms simulating a stream lake, were dosed once with the formulation Folicur® in concentrations of 5, 50, 100, 500, 1000 and 5000  $\mu\text{g/L}$  TBZ. The mesocosms were stocked with plankton, periphyton, aquatic fungi, macrozoobenthos and planted with three macrophyte species. TBZ concentrations were measured at regular intervals over an entire year (365 days) in the water and the upper sediment layer (0-5 cm) using GC-MSMS technique. The residue data were normalized to 20°C using time-step normalisation and analyzed with the R package mkin applying the five most common degradation kinetic models to the whole data set, with additional consideration of covariates like concentration or pH. For the evaluation without covariates, the combination of the single first order reversible binding degradation model (SFORB) with a two-component error model was identified to be most suitable for the degradation of TBZ.

$DT_{50}$  values derived from the slower phase of the biphasic SFORB degradation curve of TBZ in water showed a clear concentration dependence, varying between approximately 70 days for the lowest initial concentration of 5  $\mu\text{g/L}$ , and approximately 600 days for the highest initial concentration of 5000  $\mu\text{g/L}$ . Concluding, in comparison to laboratory derived  $DT_{50}$  values TBZ can be surprisingly persistent under near-natural conditions highlighting the importance of higher tier studies in regulatory science and the need for more complex decomposition models.

### **3.15.P-Mo232 Investigating the Impact of Test Conditions on Aerobic Degradation Kinetics of Aniline and Sodium Benzoate in Surface Water**

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The study evaluated the performance of aniline and sodium benzoate as reference substances for the OECD Test No. 309 (Aerobic Mineralization in Surface Water) under different test conditions. While the OECD guideline (OECD Test No. 309) recommends conducting the test at 20°C, REACH guidance for persistence assessment of PBT/vPvB substances suggest a lower temperature of 12°C. The expected time interval for degradation for these reference substances is less than 2 weeks which may correspond to 20°C. But no data is available on whether this is the case even at 12°C. Three test systems were prepared using natural surface water, which are, surface water (as such), surface water fortified with 10 mg/L suspended solids and surface water fortified with 1000 mg/L suspended solids. Each system (after spiking with the reference substance) was incubated at 20°C and 12°C each in duplicate. Samples were analyzed until complete degradation of reference substance. The natural surface water was fully characterized, including the analysis for bacterial biomass, which was measured to be  $1 \times 10^4$  colony forming units (CFU) per mL prior to treatment. In case of aniline, no significant differences were observed in degradation half-lives, which are ranging between 4.7 and 6.3 days, in all the test conditions applied. On the other hand, sodium benzoate exhibited significant differences across the test conditions. The degradation of sodium benzoate increased with higher temperatures and the presence of suspended solids. The half-lives in the surface water (as such), surface water with 10 mg/L suspended solids and surface water with 1000 mg/L suspended solids, were observed to be 3.9, 3.2, and 0.8 days at 20°C, respectively. However, the degradation rate decreased with lower temperatures (at 12°C), and the corresponding half-lives were 5.4, 4.1, and 3.0 days.

The results showed that both reference substances degraded to below detection levels within two weeks even at 12°C, indicating that both perform well in assessing the validity of the test.

The degradation of sodium benzoate was found to be influenced by temperature and the presence of suspended solids. The half-lives of sodium benzoate were shorter at higher temperatures and in the presence of suspended solids. These findings suggest that the OECD Test No. 309 can be reliably performed at 12°C and that the presence of suspended solids should be considered when interpreting the results of the test.

### **3.15.P-Mo233 High Resolution Experimental Data to Study the Remobilization and Leaching of Pesticide Residues in Vegetative Filter Strips from a Mesoscale Experimental System**

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VFSMOD, the storm-based vegetative filter strip (VFS) design model calculates runoff, sediment, and pesticide mitigation through the filters under long-term ecotoxicological regulatory exposure assessments. After each storm, the model calculates degradation of surface trapped pesticides and currently adopts a risk-conservative assumption of full remobilization for all remaining residues in the VFS surface during subsequent rainfall-runoff events. Although risk conservative, for highly-sorbed chemicals this assumption is unrealistic. As a result, an updated partial remobilization scheme was introduced in VFSMOD. We employ a mesoscale laboratory experiment as a model VFS system to validate this scheme and better understand the remobilization and leaching of surface trapped pesticide residues during a continuous sequence of rainfall runoff events. The experimental system consists of 3 vegetated soil monoliths (1.2Lx0.5Wx0.5D m) in a rain simulator. Each experiment consists

of a series of 3 rainfall events with 1 week dry period between. The series is repeated for combinations of 2 soil types and 2 seasons. Representative rainfall and field edge lateral inflow (water, sediment, tracer, and 3 pesticides of distinct sorption,  $K_{oc}$  and half-life,  $t_{1/2}$ ) inputs are controlled with >90% accuracy. Each monolith contains 12 soil probes that measure water content, electrical conductivity, and temperature at 1-min steps in a 4x3 grid along the length of the monolith. As outputs, hydrographs and breakthrough curves are collected from a surface runoff flume and 3 drainage outlets below the soil profile. Samples from each outlet are collected at 1.25-min intervals, passed through a spectral analyzer, and stored by a fraction collector. In addition, thin column soil cores are collected and divided into a vegetation and 4 soil depth samples. The water samples are analyzed for tracer and pesticide concentrations and the soil and vegetation samples are measured for pesticide concentration. From a preliminary run, the system exhibited closely the mass balance on water and tracer. Preliminary results of chemical analysis also show that there is a range of  $K_{oc}$  for which chemicals are more susceptible to remobilization, also predicted by the new remobilization algorithm in VFSSMOD. These findings illustrate the need for high spatiotemporal resolution measurements to recreate the fate and transport dynamics of a soil-plant VFS system. Plant uptake will be considered in future studies.

### 3.15.P-Mo234 The exposome and glyphosate, room for discussion

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Humans are simultaneously exposed to many chemicals known as the exposome. Although its composition is unknown, glyphosate (GLY) is likely to be one of the components in view of its ubiquitous presence in nearly all segments of the environment and in many human consumption goods.

The use of GLY is fiercely discussed since 2015 when IARC concluded in monograph 112 that GLY is "possibly carcinogenic to humans" (carcinogenicity class 2B). In contrast, certifying international bodies such as EPA, EFSA, ECHA... allow its further use. How should we deal with this dilemma?

Glyphosate is linked to negative effects on biodiversity, honeybees and on the microbiome in animals, including humans. Whether or not GLY is a carcinogen is still debated. The carcinogenicity of GLY is clearly demonstrated in vitro but carcinogenicity in real life conditions is questionable. Based on odd ratios of the risk of getting cancer after exposure to GLY, the carcinogenicity of GLY is less than the carcinogenicity of known carcinogens such as asbestos or smoking cigarettes (HGR, 2020).

After exposure to realistic doses of GLY, honeybees lose orientation during foraging and when returning to the hive while clear effects on social behavior are seen. Of note is that the acute toxic effect of GLY-preparations (e.g. roundup) is due to the adjuvant and not to GLY (Straw et al., 2023).

Killing herbs using chemicals is by definition devastating for biodiversity. However, the main reason for declining biodiversity is not (agro)chemicals but climate change and the introduction of alien species.

The effect on the microbiome is undeniable since the target of GLY in plants (the enzyme EPSPS) is also present in lower-developed organisms such as gut bacteria. Since there is a link between the microbiome and the central nervous system, disturbing the delicate balance within the enteric bacterial population is likely to affect the brain functioning.

In conclusion, GLY as part of the exposome might affect human health although the information is not complete. Application of the precautionary principle dictates the gradual and well-controlled replacement of the herbicide.

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### 3.15.P-Mo235 Novel Triple Quad Approaches for Robust and Reliable Pesticide Analysis with Ultimate Sensitivity

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Pesticides are by far the largest number of hazardous compounds analyzed in food and feed worldwide. The Rapid Alert System for Food and Feed (RASFF) network alone has reported nearly 1000 notifications of the presence of pesticides in fruits and vegetables during border control and on the market. The challenge for current technology is the number of pesticide analytes per single run. Screening techniques are becoming more popular especially for organic and pesticide-free products, while maintaining the ability to quantify the positive samples.

We propose a methodology for the analysis of pesticides in food using a novel triple quadrupole mass spectrometer. The first calibration level for most of the compounds in the study was 0.2 ng/mL in plant matrix (QuEChERS extract) with a total run time of 10 minutes (2  $\mu$ L injection). The scan time used for each compound was less than 5 ms, resulting in a method that can accommodate more than 1000 pesticides (positive and negative with fast polarity switching) with at least 2 transitions each in a single run.

Under these conditions, the calibration curve showed an R<sup>2</sup> value greater than 0.99 for 95% of the compounds included in the study. Typical ion ratios for most compounds are less than 10% of deviation. The % RSD for 10 consecutive injections is less than 10% for most pesticides in a real matrix spiked with 2 ppb of all of them. All of these parameters indicate a robust and reliable method for testing up to 1000 pesticides in a single run.

### **3.15.P-Mo236 Study on environmental behaviour of Thiamethoxam in Stringy stonecrop**

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*This study explores the behavior, dissipation, and leaching (OECD TG 312) of Thiamethoxam in three typical Stringy stonecrop (*Sedum sarmentosum*) planting soils. Dissipation patterns of pesticide residues in agricultural products were investigated during cultivation, and pre-harvest residue limits (PHRLs) were suggested based on dissipation patterns and biological half-lives. Thiamethoxam was applied to agricultural products in three different regions at the recommended dose. Samples for the dissipation pattern study were harvested at 0, 1, 3, 5, 7, 10, and 14 days after treatment, and analyzed by HPLC-MS/MS. The result indicated that Thiamethoxam dissipation followed the first-order kinetic equation in the three Stringy stonecrop samples, with similar degradation rates. The degradation half-lives in Field 1, Field 2, and Field 3 were 3.4, 3.5, and 4.0 days, respectively. For soil leaching (OECD TG 312), a PVC tube with an inner diameter of 5 cm and a length of 40 cm was used as a packed column. Dry soil (600–689.9 g) was weighed for filling, and the column was fully wetted with 0.01 M CaCl<sub>2</sub> to prepare a 30 ± 0.2 cm high leaching soil column. Thiamethoxam leaching behavior in the soil exhibited a result of 3.0 (V mobile) in the three soils. For method validation, experiments on the limit of quantitation, standard calibration curve, linearity, repeatability, reproducibility, sensitivity, and specificity were conducted. Recovery experiments validated the analytical method, satisfying criteria for pesticide residue analysis, including a recovery range of 70–110%, less than 10% relative standard derivations, and sensitivity greater than 0.01 mg/kg for the limit of quantitation or half of the maximum residue limits. The biological half-lives of pesticides in three different field studies were calculated, and PHRLs were recommended for 10 days before harvest, respectively.*

### **3.15.P-Mo237 QuEChERS Extraction and Chromatographic Determination of Selected Carbamate Pesticides in Soil Samples**

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Pesticides are generally applied to agricultural crops for protection but their residues present a potential risk to non-target organisms, affecting aquatic ecosystems and posing risks to human health. Organophosphates and carbamates impair nerve transmission in insects and poses even higher human health risks if left undetected. Recent advances have shown that these pesticides can be extracted and pre-concentrated from aqueous samples by liquid-phase micro-extraction (LPME), solid phase extraction (SPE) and headspace solid phase micro extraction (HS-SPME), while supercritical fluid extraction (SFE) and pressurised fluid extraction (PFE) can be employed for sediments. This study has shown that improved extraction allows for sensitive detection of carbamates in soil and fruit samples.

The study involves the testing of a rapid, reliable and low-solvent extraction procedure for selected carbamate pesticides of carbaryl, carbofuran, and methomyl from soil and fruit samples. It involves an investigation to determine which solvents to use, extraction optimisation, and how much of the applied carbamates are found in the fruit, compared to the soil in the same geographical location.

Preliminary results have shown that the concentrations are accumulated in the soil samples and have seasonal trends.

### **3.15.P-Mo238 Exposure Assessment for Biopesticides and Inorganics – Challenges and Improvement Needs**

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For biopesticides and inorganics, there can be significant challenges meeting the exposure assessment requirements of Reg (EU) No 283/2013 and No 284/2013. These challenges are common to several biopesticides and inorganic pesticides, and can cause uncertainties in defining appropriate risk mitigation measures and inconsistencies in product authorisations.

Recommendations for improvements, such as adapted approaches for substance mixtures are presented. This should facilitate the authorisation and integration of these pesticides into sustainable agriculture.

The data requirements for Reg (EC) No 1107/2009 are targeted primarily at discrete, single-compound, chemical pesticides. Biopesticides consist of active substances of natural origin including plant extracts (often complex mixtures), micro-organisms and semiochemicals. The environmental exposure of microorganisms and inorganic compounds is assessed generally using non-standard approaches that vary significantly from the approaches used for chemical pesticides.

Exposure evaluations of biopesticides and inorganic pesticides are often conducted by comparing expected environmental concentration with background concentrations. This requires spatially dense data which is commonly not available or too costly to acquire. To quantify exposure of micro-organisms as well as inorganics, standard approaches are simplified limiting the possibilities for risk mitigation measures. For single components of plant extracts, standard (FOCUS) approaches are generally suitable whereas problems arise for multi-component substances (often UVCBs). For latter substances, non-availability of modelling endpoints for all relevant components raises concerns. Particularly for compounds with heterogeneous compositions, defining a single representative component can be difficult and limits the identification of appropriate concepts for risk assessment. Different approaches will be presented to address these challenges, such as proposals for improving the definition of representative/lead components of substance mixtures and alternative methods to derive modelling endpoints. Also improved concepts for exposure assessment are discussed based on current experience.

There is an urgent need for adapting current methodology for exposure assessment of biopesticides and inorganics to support their role in sustainable agriculture. These advances would also help to avoid inconsistencies in decisions on authorisation of pesticides in the EU.

### **3.15.P-Mo239 Demonstrating the complexity of determining pH dependence of pesticide active substances and their metabolites in GB pesticide risk assessment**

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The dependence of degradation and/or sorption parameters with soil pH occurs when the properties - and therefore behaviour - of a substance are directly influenced by changing pH. In environmental exposure assessments, pH dependence is considered in the groundwater and surface water compartments only. Currently GB soil exposure assessments utilise the longest available DT50 (and do not consider leaching) and therefore additional consideration of pH dependence is not required for this compartment.

The two most likely reasons that the properties of a substance are directly influenced by pH are either that the substance is ionisable, or that it is prone to hydrolytic breakdown. It is important to follow the following 3 steps to determine whether pH dependence is relevant:

1. Establish if there are good scientific or **mechanistic** reasons to suspect that a relationship exists
2. Investigate the significance of the correlation using adequate **statistical** tests
3. If steps 1 and 2 indicate pH dependence, the exposure assessment should be performed accounting for any significant correlation.

Where contradictory information exists, a weight of evidence approach should be observed to determine the inclusion of pH effects in the exposure assessment. This is because if there is a plausible mechanistic basis for pH effects, supported by at least one of the statistical analyses, possible pH effects should not be discounted from the exposure assessment based on the results from the statistical tests. Determining statistically robust relationships between substance and soil properties can be difficult when dealing with small data sizes.

The subsequent exposure assessment accounts for pH dependence by undertaking modelling calculations using substance parameters derived from different agriculturally relevant acidic and alkali soil pHs. By modelling the active substance behaviour across a variety of pH dependent soil parameters (such as DT50 and Koc), the worst case PECs can be calculated based on this combination of factors.

This poster aims to demonstrate the decision making process when using mechanistic and statistical methods for concluding pH dependence in pesticide active substance exposure assessments.

### **3.15.P-Mo240 Proposed revision of the aged-sorption guidance document in combination with field degradation studies in regulatory assessments**

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In the EU Aged Sorption Guidance the use of field DegT50 values in combination with aged sorption parameters is only possible to a very limited extent. If the leaching assessment for a plant protection product (PPP) is based on field DegT50 values only, it is not recommended by the current guidance to derive a DegT50<sub>equilibrium</sub> (rescaling of DegT50) for input in exposure models in combination with aged sorption parameters ( $f_{NE}$ ,  $k_{des}$ ) as described for laboratory DegT50 values. This virtually prevents the mitigating effect of aged sorption on leaching and limits the applicability of the aged sorption guidance in combination with field DegT50 to very few cases.

However, the guidance mentions that “Industry is preparing evidence for aged sorption in field studies and this option should replace the current recommendations as soon as appropriate guidance has been developed and tested”.

Therefore, additional field studies were evaluated to show the relevance of aged sorption in the field. The evaluation was mainly based on the comparison of observed concentration depth profiles versus FOCUS-PEARL simulations with and without consideration of aged sorption. Where possible, site-specific aged sorption parameters from laboratory studies with the respective field soils were used. The depth to which 95% of the residues are found was defined as metric [P5 metric] for comparison.

Proposed revisions to the current EU Aged Sorption Guidance fully allowing for the combination of field degradation and aged-sorption data are presented.

### **3.15.P-Mo241 Can Drainage Losses be Reduced by Optimizing Pesticide Application Dates?**

*Prakash Srinivasan<sup>1</sup>, Carolin Kley<sup>2</sup> and Dieter Schaefer<sup>2</sup>, (1)Bayer AG, (2)Bayer AG - Crop Science Division, Germany*  
Presently, in the EU risk assessment for plant protection products, there are no mitigation or refinement options available for drainage exposure apart from reducing the intended application rate and generic application time restrictions. One more flexible mitigation option could be to look in more detail at the application timing to omit the application at dates which lead to highest potential exposure via drainage. Recently published monitoring data (Tournebize et al., 2023) from the La Jaillièrè experimental site in France has revealed that drainage losses remarkably increased when soil moisture exceeded a certain threshold at the time of pesticide application, thereby increasing the pesticide concentrations in the receiving water bodies.

This study examines the role of soil moisture on the application day as a potential trigger in influencing exposure via drainage. Existing FOCUS and UK drainage scenarios were used to calculate a soil moisture trigger value in terms of the soil wetness index (SWI). Limiting the pesticide application to periods when the SWI is below this trigger value could reduce environmental safety concerns and enable safe uses with respect to drainage entries into water bodies. Three herbicides were chosen for this assessment. Step 3 MACRO runs were simulated with no interception and with additional hydrological outputs being switched on needed for calculating SWI on the day of application. Predicted environmental concentrations (PEC) in ditch and stream were calculated using TOXSWA model with no drift entry. The resulting maximum PEC/RAC ratio for a 1-year period after application was plotted against the SWI on the day of application.

The results show that for herbicide C for scenario D2 Brimstone a potential trigger could be set at 60% SWI. Herbicide B for scenario D6 Thiva has a potential trigger of 85% SWI. For D5 La Jaillièrè, for herbicide A, B and C, no clear threshold trigger was observed with 10% of the data points not following the pattern observed in the monitoring study; excluding these values, a potential SWI trigger of 85% could be observed.

It can be concluded that the threshold triggers and the magnitude of their effect on drainage losses varied between scenarios and substances depending on their properties. Thus, the decision about the applicability of this approach as a potential drainage mitigation must be done on a case-by-case basis.

### **3.15.P-Mo242 A Workshop Discussion on Options for Mitigation of Drain Flow and Groundwater Contamination by Pesticides**

*James Garratt, Adam Doxford and Charles Hazlerigg, Enviresearch Ltd., United Kingdom*

There are relatively few options available for mitigating the risk for drain flow or groundwater in the UK. The usual approach is to reduce the application rate or change the timing. The UK Health and Safety Executive (HSE) funded a project to explore additional options for mitigation. The focus of this project was an online workshop to gather the views of various stakeholders from product development through to land management.

There were 38 external participants in the workshop, plus 10 participants from the project team and from the UK authority (HSE). The workshop was structured into three parallel sessions that covered ‘Land Management’, ‘Precision Agriculture’ and ‘Formulation Development’. The conversations were moderated to encourage engagement but not to direct the responses. The goal was free-flowing discussion that enabled all ideas to be aired. Following a review of the workshop output, we suggest the following measures as promising new solutions, evolutions of existing solutions or furthering those currently in development.

- *New label phrases* with restrictions based on identifiable features of the field (e.g. the presence of field drains, particular soil types), coupled with Geographical Information Systems to ensure compliance.
- Inclusion of the *Scale of Use* in the regulatory regime could take a more proportional approach to risk mitigation of groundwater, and to some extent drain flow, for minor crops.
- *Better quantification of Precision Application* could give a more accurate representation of pesticide applied to a proportion of the field.
- In a *2-staged Application Process*, ‘test cases’ could be considered by HSE before the main evaluation, reducing the perceived risk by applicants in taking new approaches to a full evaluation. Linked to this, better guidance to support *Non-standard Assessment* methodologies.



- The integration of *Water Monitoring* into the authorisation process would allow quantitative assessment of the impact of any new measures or changes to the risk assessment.
- A consideration of *Adjuvant Risk Assessment* could permit authorisation of a formulation when applied with an adjuvant that reduces the exposure/risk.
- A measure of *Rainfastness* has the potential to refine the assessment to give a more accurate representation of what has adhered to the crop, thus reducing overall exposure.
- *Decision Support Systems* used by land managers could be considered for possible integration into risk management tools.

### **3.15.P-Mo243 Landscape level modelling for derivation of worst-case surface water dilution factors at drinking water abstraction locations**

*Mohammad Hatamjafari, Sebastian Gebler and Tom Schröder, BASF SE, Germany*

EFSA issued a guidance document on the impact of water treatment processes on residues of active substances or their metabolites in water abstracted for the production of drinking water. The guidance contains recommendations for the step 1 screening process, the exposure assessment of plant protection products at drinking water (DW) abstraction locations, originating either from ground water or surface water (SW). For drinking water abstracted from surface water, the guidance proposes calculating predicted environmental concentrations (PEC) at drinking water abstraction locations (PEC<sub>dw</sub>) by applying a dilution factors (DF) to edge-of-field PEC<sub>sw</sub> calculated according to FOCUS (FORum for the Co-ordination of pesticide fate models and their USE). Worst-case DF are to be used as first tier; DF derived from GIS-based landscape-level assessments can be used as higher tier options. This study is an extension and continuation of a previous geospatial analysis that builds upon the Dutch DROPLET model and investigated generic dilution factors starting from edge-of-field PEC<sub>sw</sub> and extending to a potential drinking water abstraction location downstream at the catchment outlet. This innovative approach incorporates agricultural land use and hydrology considerations and utilizes a stepwise GIS-based approach at EU level to include the impact of further environmental parameters that determine the environmental fate of chemical substances. The present study explores the significance of upstream agricultural land use, catchment hydrology, and connectivity of treated fields in relation to the proposed datasets and parameters outlined in the guidance, as well as beyond. This includes the use of higher resolution state-of-the-art land use data together with a more granular catchment and stream network for all EU regulatory zones. Results show a significant difference with the proposed worst-case dilution factors in the guidance. The methodology and used datasets contribute to a more realistic step 1 screening process.

### **3.15.P-Mo244 Drinking Water Treatment – Impact Assessment on a Newly Guided Process for Crop Protection Products in the European Union**

*Kristina Ziegler and Lydia Pape, knoell Germany GmbH, Germany*

After years of uncertainty how to address the data requirements for drinking water treatment processes under the Regulation (EC) No 1107/2009 the European Food Safety Authority (EFSA) published in August 2023 the final ‘Guidance document on the impact of water treatment processes on residues of active substances and their metabolites in water abstracted for the production of drinking water’.

The new assessment framework introduces an interdisciplinary tiered approach which should reflect the real situation regarding drinking water treatment in the EU and should allow for a conclusion on concerns for public health from exposure to harmful by-products in drinking water. Starting with an environmental fate perspective, novel compounds potentially evolving from known residues in groundwater and surface water (active substances and their environmental metabolites) during the water treatment processes shall be identified and assessed further.

For understanding the outline and potential consequences of the guidance document from an environmental fate and modelling perspective, an impact assessment was initiated. Publicly available data on active substance approval level will be searched and evaluated on the success of current attempts to address the data requirement on drinking water treatment in the area of crop protection. Further, gathered information will be consolidated to allow for conclusion on common approaches, e.g. for structurally similar compounds.

Many previously submitted assessments focused mainly on chlorination and ozonation. This will not be sufficient anymore as the new guidance document additionally considers the processes of UV disinfection and sand filtration. In this context, the possibility to make use of already existing approaches and options for data cross-reading will be evaluated to avoid extensive experimental laboratory testing, where applicable.

Conclusively, the presentation will highlight the challenges related to the EFSA guidance document on the impact of drinking water treatment processes from a crop protection perspective and will provide conceptual strategies for solutions.

Keywords: crop protection, active substance approval, drinking water treatment, chlorination, ozonation, UV disinfection, sand filtration, water treatment transformation products

### 3.15.P-Mo245 Groundwater Dilution Factor Evaluation in the EU using the Spatial LUCAS PEARL Modelling Framework

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EFSA and ECHA have published a new Guidance on the impact of water treatment on residues of active substance or their metabolites in water abstracted from surface water or groundwater for drinking water production. In this guidance, the exposure assessment of Plant Protection Products (PPPs) at drinking water abstraction locations from surface water considers an attenuation effect during the fluvial transport process. This is achieved by introducing a dilution factor (DF) which varies for different regulatory zones in the EU. However, the guidance does not propose a DF for the exposure at drinking water abstraction locations from groundwater. Instead, it assumes that the simulated Predicted Environmental Concentrations in groundwater at a soil depth of 1 meter ( $PEC_{gw,1m}$ ) will serve as the final exposure indicator for drinking water abstraction evaluation. The attenuation of PPP concentrations along the flow path from the regulatory  $PEC_{gw,1m}$  to raw groundwater abstraction location has been scientifically discussed on a catchment scale. However, peer-reviewed literature does not provide a systematic study on the groundwater DFs estimation on the EU level. Comprehensively quantifying groundwater DFs, by considering both vertical and horizontal aquifer attenuation processes, poses a challenge due to the limited availability of geohydrological data at the EU level.

The new Guidance on drinking water treatment suggests that a depth of 1–2m below the shallow groundwater table (i.e. GWT < 10 m) can be used as a conservatively representative depth for aquifer quality evaluation. In this work, we investigate the EU-level groundwater DFs from  $PEC_{gw,1m}$  to  $PEC_{gw}$  at this suggested groundwater depth (i.e.  $PEC_{gw, GWT+1-2m}$  for GWT < 10m) by considering vertical attenuation processes only. For this the LUCAS spatial soil data was coupled with the 1D leaching model FOCUS-PEARL 5.5.5. Dummy compound experiments in the LUCAS PEARL modelling framework will be used for quantification of groundwater DFs from the 1m soil depth towards the 1-2m below GWT depth (GWT < 10m) for the whole EU. Additionally, the impact of compound properties on groundwater DFs will be explored through varying the values of half-life degradation ( $DT_{50}$ ) and adsorption distribution ( $K_{oc}$ ) parameter in the dummy compound experiments. The work will contribute to fill the knowledge gap of groundwater DF estimation for the regulatory groundwater exposure assessment for drinking water production on EU level.

### 3.15.P-Mo246 Sensitivity of Water and Sediment Concentrations Simulated by the TOXSWA Model

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The TOXSWA model simulates exposure concentrations for the aquatic risk assessment as part of the Dutch and EU pesticide registration procedures. We present initial results of a sensitivity analysis of TOXSWA for surface water systems with dynamic flows such as the EU FOCUS ponds, ditches or streams.

The aim of the analysis is to assess which input parameters contribute most to the studied target output parameters, esp. the peak  $PEC_{wat}$  and  $PEC_{sed}$  (Predicted Exposure Concentrations in water and sediment). We first studied the effect of the pesticide properties input for the FOCUS R1 pond. The sampled pesticide properties originate from the Pesticide Properties DataBase.

We used the sampling method consisting of Saltelli's extension of the Sobol sequence to calculate the  $S_1$ ,  $S_2$  and  $S_T$  indices, i.e. the first-order, second-order and total-order Sobol indices.  $S_1$  measures the contribution to the output parameter's variance by a single model input alone,  $S_2$  the contribution by the interaction of two model inputs, for all possible combinations, while  $S_T$  measures the contribution by a model input, including both its first-order effects and all higher order interactions. We did around 50 000 model runs.

The  $S_1$  indices for the R1 pond show that a single parameter accounts for 89% of the variation in the  $PEC_{wat}$ : the  $K_{om,ss}$ , the coefficient for Freundlich sorption to suspended solids. The next most sensitive input parameter is the  $K_{mp}$ , the coefficient for linear sorption to macrophytes, with a  $S_1$  index of only 2%. This is significantly lower than expected based on field experiments. Very probably, this is related to the limited input range of the  $K_{mp}$ , compared to the one of the  $K_{om,ss}$ . Concerning the  $PEC_{sed}$  the three highest  $S_1$  indices are 34% for the  $K_{om,sed}$  (Freundlich coefficient for sorption to sediment) and 6% for the degradation half-lives in water and in sediment. Their  $S_T$  indices raise to 63%, 21% and 18%, indicating that significant interactions with other input parameters exist. The sum of the  $S_T$  indices for all studied input parameters is 147%, indicating that the TOXSWA model behaves non-linearly with respect to the  $PEC_{sed}$ .

A next step is to look into the variation of the  $PEC_{wat}$  and  $PEC_{sed}$  for the FOCUS R1 stream with its high flow velocities. The results of the sensitivity analysis allow for a better understanding of the TOXSWA model behaviour, which is useful for model validation and for definition of e.g. sediment scenarios for use in registration.

### 3.15.P-Mo247 Reduction of Complexity: Variance-based Sensitivity Analysis for FOCUS STEPS

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To standardize the role of models in the European Union (EU) plant protection product authorization process, Forum for the Co-ordination of pesticide fate models and their use (FOCUS) has established guidelines, including the FOCUS STEPS models

for surface water risk assessment. Conservative scenarios representing EU member states are used, with a 4-tier approach for limit value exceedance. Higher-tier calculations, while requiring more information, provide more accurate results. When calculating the respective tiers, however, the accuracy of the model parameters does not have to be specified. This can mean that a parameter to which the model reacts very sensitively - i.e. the result depends relatively heavily on variations of it - can lead to a large inaccuracy.

The project employs sensitivity analysis using the Sobol' method. Model parameters are sampled using low-discrepancy sequences, and Sobol' indices are calculated to determine the sensitivity of the FOCUS STEPS models to parameter variances. The analysis focuses on the first order Sobol' indices representing the main effects, providing insights into parameter influence.

FOCUS STEPS 1 as the simplest example, is presented with a limited set of parameters. The analysis of scenario parameters, such as drift and runoff proportions, field-to-water ratio, and water depth, reveals sensitivity patterns. For low values of the sorption constant  $K_{oc}$ , the model is sensitive to runoff parameters, while higher  $K_{oc}$  values make it more sensitive to drift fraction. With low  $K_{oc}$  leading to high concentrations, the variance of runoff parameters should, hence, be decreased to a minimum. Higher Tiers are handled to some extent afterwards.

Sensitivity analyses help identify influential processes and parameters. The variance-based analysis guides users on reducing uncertainties, especially when concentrations are near regulatory thresholds. Considerations for modifying model user interfaces to suggest variance sensitivity based on input configurations are proposed for enhanced user guidance.

### **3.15.P-Mo248 The Effect of Time-Varying Soil Properties Caused by Ploughing and Consolidation on Pesticide Fate in Soil and Groundwater**

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Soil properties such as the dry bulk density and soil hydraulic parameters can significantly affect the environmental fate of pesticides. These properties are often assumed to remain constant in time in numerical models. However, these properties change over time during ploughing and consolidation in reality. In this study, we modeled the time-varying soil properties induced by ploughing and consolidation and assessed its effect on pesticide accumulation in the topsoil and leaching to the groundwater. For this purpose, time-dependent soil properties have been implemented in the pesticide fate model PEARL. Ploughing instantaneously decreases the bulk density, after which it gradually increases again to its original value through consolidation caused by rainfall. The time-dependent soil properties are modelled based on empirical relationships between the dry bulk density and the Mualem-Van Genuchten parameters found in the literature.

Ploughing leads to a short-term deviation of the soil water content and concentration compared to the reference case (i.e., the case with constant soil properties). We included mixing of pesticide over the ploughing layer due to ploughing in both cases. However, under Central European climate conditions, the effect of ploughing vanishes within several months in the entire soil profile. For assessing the impact on the leaching of pesticide to groundwater, we evaluated the pesticide concentration in pore water at 1 meter depth. The effect of time-varying soil properties due to ploughing and consolidation on the leaching concentration was found to be small for both a tracer and an adsorbing solute. Even for an extreme case with three ploughing events per year, the effect on the 90<sup>th</sup>-percentile of daily leaching concentration was smaller than 0.3%. For assessing the impact on the exposure of soil organisms to pesticides, we considered the pesticide concentration in pore water averaged over the upper 20 centimeters of the soil. For the tracer, ploughing resulted in a 1.2% decrease of the 90<sup>th</sup>-percentile of daily topsoil concentration data for the extreme case of three ploughing events per year. Interpretation of the results for adsorbing solutes in the topsoil is hampered by the fact that soil mass is not conserved in the current approach. More advanced models must be developed that allow for conservation of soil mass for assessing the impact of time-dependent soil properties on concentrations in the topsoil.

### **3.15.P-Mo249 C2D2 Novel Crop Development Data for Use in Pesticide Exposure Modelling and Risk Assessment**

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Determining appropriate pesticide application windows is an important part of pesticide exposure modelling. Increasingly, regulators are constraining application windows using the AppDate software. Given exposure assessments are a function of the application dates this software generates, it is important to understand how representative they are; however, an appropriate pan-European crop phenology dataset was previously unavailable for such an assessment. Efficacy trial datasets sourced from seven members have been harmonised to create the Crop Life Europe Crop Development Database (C2D2) that meets this need and has been made freely available under a Creative Commons licence (<https://zenodo.org/records/8393135>) to facilitate innovation in exposure science to allow for more accurate and realistic risk assessment leading to enhanced crop and environmental protection.

The C2D2 database holds data for a wide range of crops, including some crops that have a limited geographic distribution and extent, for example cotton. Another such crop is rice which has very specific production requirements and is only produced in a small number of locations within the EU. While rice is not covered by the FOCUS<sub>sw/gw</sub> scenarios, exposure scenarios to assess the fate of active substances applied to this crop are available and evolving. A comparison of the rice data held within C2D2 was made against the proposed scenario crop development dates for the four scenarios covering Greece, Italy, Spain and France/Portugal. The analysis indicates that there is sufficient C2D2 rice data in Spain and Italy that a comparison can be undertaken and indicates that the proposed crop development is plausible albeit there is room for refinement. This assessment illustrates how crop development stages for novel and/or minor crops that are not covered by the existing FOCUS<sub>sw/gw</sub> scenarios might be developed using C2D2. While previous C2D2 publications have focussed on the widespread combinable crops, this poster explores how C2D2 might be used for other novel and minor crops, for example nuts, citrus, cotton, tobacco and lavender.

### **3.15.P-Mo250 The role of the ratio of sorption to degradation in leaching**

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Models are used extensively to predict the leaching of pesticides. For example in the EU, PEARL, PELMO, and MACRO are numerical models used in the regulatory risk assessment. These models are often complex in that they consider many processes (e.g. temperature and water distribution within the soil, crop growth, nonlinear compound sorption, and compound transport due to advection, diffusion, and dispersion). The purpose of regulatory models is to be a conservative indicator of the potential for leaching, and it is important that these models continue to incorporate advancements in mechanistic understanding.

Whilst scientific development of these models is necessary, it is also paramount to understand the relative importance of the processes considered. There is a risk that ever-increasing complexities associated with pesticide transport in soils causes models to be treated as black boxes and fundamental understanding is lost. In this work we will use mathematical methods to highlight the role of the ratio of sorption and degradation in compound transport.

To derive fundamental insight, we extend the work of Jury (1987) and analytically derive the steady state solution for pesticide transport as a function of depth considering nonlinear sorption using the mathematical technique of asymptotic expansions. We consider that degradation occurs in both the soil and liquid phases and that transport is driven by advection, diffusion, and dispersion. We include the influence of temperature on biodegradation, and the role of depth on sorption and biodegradation. We compare the steady state analytical solution with extended duration PEARL runs for all FOCUS scenarios and find that the ratio of sorption to degradation is generally the dominant factor in leaching. However as absolute values of degradation and sorption decrease, the influence of weather fluctuations increases.

In summary, we present an analytical solution of the steady state of pesticide leaching that considers nonlinear sorption and biodegradation in all soil phases. We show the importance of considering the ratio of sorption and degradation as a dominant determinant in pesticide leaching rather than independent properties. Lastly, whilst this method is applied here to PEARL and FOCUS scenario weather files, this method can be applied to any region where chromatographic flow is relevant, and this method allows either simple or complex parameterisation (i.e. from average conditions to weather files).

### **3.15.P-Mo251 MACRO 5 Multithreading for the CRD UK higher-tier drainflow tool**

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Introduction: A software tool was developed that allows the application of MACRO 5 multithreading to the official HSE CRD UK higher-tier drainflow tool. The current UK higher-tier drainflow tool allows sequential runs of MACRO 5, i.e. it is based on a "single run" MACRO 5 approach, resulting in high simulation times for all 12 scenarios.

The MACRO 5 multithreading tool is an add-on to the HSE CRD UK higher-tier drainflow tool v2.1. The add-on was developed in collaboration with the UK pesticide authority HSE. The purpose of the multithreading tool is to reduce the simulation time of the MACRO 5 runs by allowing multiple MACRO runs in parallel instead of running MACRO sequentially.

Technical details of the tool and benefits for the UK higher-tier drainflow assessment are presented and discussed.

Materials and methods: The multithreading software tool was developed in C# as a standalone GUI. The tool is able to run the 12 scenario/climate combinations in parallel. It is able to run all three types of MACRO runs (Parent, Driving files and Metabolite). Evaluations are still possible with the HSE CRD software tool. In addition, the multithreading add-on tool can be opened in multiple instances. Therefore, two or more separate substances with e.g. 12 scenarios each, could be run in parallel, only limited to the processor power in a computer. Test runs were performed with different setups and total number of MACRO runs.

Results and discussion: The calculation time decreased significantly for MACRO 5 by ~80 to 90% using multithreading. If metabolites are considered, the simulation time would increase for sequential runs because of additional intermediate and metabolite runs.

Conclusions: In conclusion, the multithreading software tool has the following properties:

- Standalone GUI
- Multithreading allows a reduction of MACRO 5 calculation time by around 90%
- No adaptation or manipulation of the existing MACRO model necessary, no changes in program code
- Official add-on to the official UK higher-tier drainflow tool, to be released via HSE CRD website

Future implementations

- A standalone MACRO multithreading GUI for the current FOCUS SW and GW software is already available
- Implementation into the current FOCUS software suite possible without any software adaptation or model changes
- GUI for FOCUS SW multi-year approach would be also available soon
- Discussion about implementation with FOCUS VC and EFSA necessary

### **3.15.P-Mo252 Acre Tool For Automated Surface Water Modelling**

*Alice Tagliati, Wendy van Beinum and Scott Watson, Enviresearch Ltd., United Kingdom*

Acre is Enviresearch's automation software designed to aid the agrochemical industry in modelling the fate of pesticides in the environment. Using the same FOCUS models and scenarios as the official FOCUS tools, Acre offers a low effort solution for conducting surface water modelling to support preliminary aquatic risk assessments for new products, uses, and product renewals.

It streamlines the surface water assessments for pesticides by automating the FOCUS Step 3 and 4 surface water modelling process. With Acre, modelling a large set of scenarios that would typically require several days to run manually can be performed in a matter of hours.

- All inputs necessary for running the FOCUS Step 3 and Step 4 simulations within a single user-friendly spreadsheet. This eliminates the need to navigate various interfaces for setting up simulations and reducing the risk of human error from input of inaccurate information.
- Enables high-throughput running simulations, allowing multiple substances, crops, pesticide application rates and timings, and mitigation combinations to be managed within a single spreadsheet.
- Low effort solution for scoping new products with a large set of potential uses and mitigations across various countries in the EU.

Enviresearch is looking for regulatory acceptance of automated modelling tools such as Acre to be recognized and accepted alongside the official tools overseen by FOCUS version control. Acre replaces the FOCUS shells by controlling the input and execution of the models. It is therefore essential that Acre is taken through the same level of scrutiny and testing as the FOCUS tools. Whilst automation is becoming the norm, there is a general need within the regulatory community to address the process for regulatory acceptance of new tools, and how to manage testing and version control.

### **3.15.P-Mo253 Development of an Interim Modelling Methodology using the FOCUS Models for Simulating the Fate of Copper in Soil, Water and Sediment**

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The 2021 EFSA PPR Statement on environmental risk assessments for transition metals indicates that exposure models and scenarios would need to be adapted to address environmental processes and parameters relevant to the fate and behaviour of transition metals in water, sediment and soils.

This study on behalf of the EU Copper Task force seeks to identify shortfalls in the current suite of FOCUS pesticide fate models for simulating the fate of copper in soil and water and develop an interim modelling methodology with the FOCUS models to address those shortfalls until a full modelling framework for transmission metals is available.

Processes common to copper and organic plant protection products and present in the FOCUS MACRO, PEARL or TOXSWA models were identified e.g. equilibrium sorption to soil solids, non-equilibrium sorption to soil solids, equilibrium sorption to suspended sediments, diffusive exchange between water column and bottom sediments and equilibrium sorption to macrophyte surfaces.

Processes important for copper not present in MACRO, PEARL or TOXSWA were also identified e.g. fixation of copper into soil and sediment solids, soil erosion and transfer of copper to surface waters, deposition and resuspension of sediments in surface waters, dissolution of solid copper forms and formulations following deposition onto plant surfaces, soils and surface waters, complexation in surface water, influence of the presence of a natural background of copper and formation and dissolution of copper sulphide in anoxic sediment layers.

Methodologies were developed to implement important copper processes within the current FOCUS models through parameterisation of surrogate processes included in the models. Processes such as dissolution of particulate copper formulations were simulated as a parent (solid) to metabolite (dissolved) relationship in soil, water and sediment using dissolution study data while degradation in soil served to simulate ageing of copper in soil and sediment.

The modelling approaches developed retain the FOCUS modelling framework and scenarios we know to provide an acceptable description of agronomically relevant hydrological scenarios and simulate important processes for describing the fate of copper in soil, water and sediment, allowing for risk assessment of environmentally relevant and available dissolved copper as an interim modelling approach until such time as bespoke models and frameworks are available for transmission metals.

### **3.15.P-Mo254 Development and validation of a hybrid GIS catchment vulnerability ranking model for losses of pesticides to surface water**

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The intensive use of pesticides has led to their widespread presence in the environment. Although pesticides are primarily applied to crops or soil, various transport mechanisms contribute to elevated pesticide concentrations in surface waters. To comprehend the potential sources and pathways of pesticides to surface waters, detailed monitoring and modelling studies have been undertaken. To extrapolate the insights gained from these studies to other locations, it is crucial to contextualize these catchments and their vulnerability to pesticide presence in surface water. The development of a catchment vulnerability ranking index is essential in this regard, serving as a decision-making tool to prioritize catchments for future studies or to identify locations where mitigation measures would be most beneficial.

A catchment vulnerability ranking method was developed for the presence of pesticides in surface waters. This method has been applied to Flanders, Belgium, a region characterized by frequent exceedances of pesticide environmental limits in surface waters. The vulnerability of catchments is assessed based on a hybrid GIS approach, considering pesticide emissions through two primary transport routes: surface runoff and soil erosion. Pesticide emissions are calculated at the parcel-level considering environmental factors such as land use, crop rotations, soil characteristics, precipitation, and topography, as well as the chemical properties and usage patterns of the selected substances. Pesticide emissions are evaluated at the catchment level, thereby explicitly accounting for landscape connectivity.

Leveraging data on discharge and pesticide concentrations from a monitoring network in Flanders, pesticide loads were calculated at 15 locations. An automated point-detection algorithm was applied and only observations during the application period were considered for load calculations. These loads and their associated catchment vulnerabilities served as a validation dataset for the developed method. Initial findings indicate a good agreement between observed and modeled loads, particularly when considering inputs from erosion alone or a combination of erosion and runoff.

The application of this method to the entirety of Flanders will empower policymakers to readily identify catchments prone to pesticide presence in surface waters and to guide them in selecting priority locations for monitoring and implementing mitigation measures to safeguard water quality.

### **3.15.P-Mo255 Modeling the Exposure of Soil Organisms to Pesticides in Brazil: A Scenario for Tier-1**

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Pesticides, widely used in agriculture, can pose risks to soil organisms and ecosystem functions. Thus, it's crucial to conduct a tiered Environmental Risk Assessment (ERA) of pesticides in soil, starting with a conservative and simplified scenario (Tier-1) and progressing to more complex scenarios. However, the ERA for soil organisms in Brazil is yet to be implemented.

This study proposes a Tier-1 exposure scenario for soil organisms in Brazil, based on a geospatial analytical model. This model estimates the environmental concentrations of pesticides in soil using spatially explicit data on soil properties, temperature, and agricultural land use.

The model applies first-order kinetics with temperature adjustment and a Q10 factor to calculate the predicted environmental concentration (PEC) for each day of the year, over 10 years, assuming a constant application rate of 1 kg a.i. ha<sup>-1</sup> on the first

day of each year. Five half-life values ( $DT_{50} = 10, 31, 100, 316, \text{ and } 1000$  days) were tested to represent different levels of pesticide persistence in soil.

The model considers first-order kinetics, temperature adjustment, and conservative assumptions, such as no interception, retention, or removal processes of pesticides in soil. It estimates the PEC of pesticides in soil for each pixel of  $1 \text{ km}^2$  and each half-life time, using geospatial data of soil density and daily mean temperature.

The 90th percentile ( $P_{90}$ ) of the PEC distribution was used as the reference parameter for the exposure assessment. The location and characteristics that best matched the  $P_{90}$  value were identified and used as the Tier-1 exposure scenario for soil organisms in Brazil. This scenario is located in the south of São Paulo state, on a Latossolo Vermelho-Amarelo (Oxisol) of sandy clay texture with low organic carbon and CEC, and a mean daily temperature ranging from  $15.6$  to  $24.3 \text{ }^\circ\text{C}$ .

The scenario method is easy to apply on ESCAPE 2.0, providing a conservative estimate of the PEC for Tier-1 ERA. It can also be adapted to other  $DT_{50}$  values and exposure patterns and can be refined in higher tiers using crop-specific dependent scenarios or numerical models. This method can help advance the implementation of the ERA for soil organisms in Brazil, supporting the decision-making process for pesticide registration and management.

### **3.15.P-Mo256 Promoting Defensible Science in Aquatic Exposure Estimation by Integrating Landscape-Level Data into US Endangered Species Assessments**

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Prospective aquatic exposure modeling is a key component of the assessment of potential jeopardy of endangered species during the preparation of a risk assessment related to federal US pesticide (re)registration. The “Biological Evaluation” prepared by the US Environmental Protection Agency (USEPA) provides the foundation of aquatic exposure estimates using well-established pesticide fate and transport models applied to a standardized set of crop/soil/weather scenarios. Exposure estimates are based on 30 years of model simulation representing labeled uses of the pesticide assuming maximum application rates, maximum number of applications, and minimum re-application intervals, as well as assuming treated crop is adjacent to the aquatic environment. This modeling forms the basis from which subsequent refinements incorporating spatial, temporal, and pesticide usage variability can be applied. Further refinement of the screening-level modeling is essential to more accurately inform the weight of evidence process used by the US Fish and Wildlife Service and National Marine Fisheries Service to determine species jeopardy in the next stage of the assessment, the “Biological Opinion”. To support this refinement, we present a highly efficient, quantitative approach that builds on the USEPA’s aquatic modeling by increasing the spatial/temporal context and resolution of exposure estimates to species of interest and producing well-defined and reproducible species-specific estimated aquatic concentrations. Utilizing data for each species, a set of high-resolution catchments, landscape-based crop proximity and density, and pesticide usage, inputs were processed to produce landscape-level exposure concentrations suitable for aggregation at multiple spatial scales appropriate for the species being examined. These refinements show that aquatic concentrations based on screening level assumptions of pesticide use and hypothetical water body scenarios may occur at some locations at limited times, but they are far less likely to occur within species range/habitat than is assumed in the screening-level risk assessments. The methodologies presented are a quantitative approach to refining aquatic exposure estimates that incorporate variations in landscape and agronomic practices near endangered species locations and provide essential information to help inform localized mitigations for individual species across specific use patterns.

### **3.15.P-Mo257 Patterns in agricultural practice related concentrations of currently-use pesticides in soils from the province Friesland, Netherlands**

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Farmland birds are often of sensitive conservation status. One of the stressors that may result in their population size decline, is that these birds depend on areas where often multiple pesticides are used. In 2020, 5 million killogram of pesticides were applied on Dutch agricultural fields and a total of 257 active substances are approved for usage in the Netherlands. Despite this wide variety of pesticides and their large-scale application, a potential direct correlation between pesticide usage and the decline in farmland birds remains poorly studied. As a first step to achieve a better understanding of the potential exposure of farmland birds to pesticides, our current study aims to quantify currently-used pesticides that are present in soils of farmland birds’ habitats, and relate these observed pesticide concentrations to current and historic land-use. Soil samples were collected from 26 different agricultural fields in June and July 2020 in the province Friesland (Netherlands), which is an essential breeding area of the farmland bird *Limosa limosa*. The sampled plots consisted of: i) 4 bulb fields, ii) 7 corn fields, iii) 8 grassland fields, and iv) 7 old-grassland fields. Old-grassland means that, as far as known, no other crop than grass has been cultivated on the field. 15 samples were randomly obtained (0-5 cm depth) from each plot and pooled into 3 composite samples per plot. All soil extracts were screened for 648 pesticides using LC-MS/MS, detected pesticides were quantified and the derived concentrations correlated to current land-use, historic land-use, and agricultural practice. Not all derived pesticide concentrations are in line with expected patterns about pesticide usage mentioned in literature. An explanation is that our current study does not cover different seasons or time points, while time is a decisive factor. Although work is still in progress,

we can already conclude that: i) the general pattern is that bulb and corn fields contain the highest concentrations of pesticides, ii) the lowest concentrations and lowest number of pesticides were observed in old-grassland fields, iii) the number and pesticide concentrations in grassland are dependent on the land-use history of a plot, and iv) over time, pesticide concentration levels resulting from bulb cultivation, decrease. However, pesticides used for bulb cultivation may still be observed years after ending bulb cultivation.

### **3.15.P-Mo258 Accelerated Solvent Extraction with Liquid Chromatography Targeted Tandem Mass Spectrometry and Nontargeted High Resolution Mass Spectrometric Methods as a Tool for Targeted Pesticide Management**

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Using pesticides to ensure high crop yields is integral to modern agriculture. However, with increased productivity comes growing concern about the potential impact of pesticides and their metabolites on the environment and human health through food and groundwater.

Herbicides are the most used class of pesticides in the Czech Republic. However, they are usually applied to crops like rape, corn and sugar beets. This work focused on 14 agricultural fields in the Czech Republic in July 2023, mainly used for fast-growing and highly variable crops like vegetables. Out of 110 pesticides and their metabolites analyzed by the targeted LC/QqQ method, 73 were above the LOQ in at least one soil. Fields with the same crops showed highly variable contamination patterns. Only one field with carrots was polluted with bentazone (320 ng/g) and linuron (140 ng/g). In other samples, those compounds were not detected or were 2 orders of magnitude lower.

On the other hand, azoxystrobin, a fungicide widely used in agriculture, was detected in all tested fields (in carrot fields 22-1600 ng/g; potato fields 15-720 ng/g; onion, lettuce, and parsley fields- 220-340 ng/g). The targeted method used for analysis was rooted in the range of pesticides and their transformation product frequently found in surface and ground waters. Despite some overlaps, e.g., azoxystrobin, epoxiconazole and tebuconazole, we hypothesize that soil pesticide patterns should differ from surface water. We applied nontargeted LC – full scan HRMS with a data-independent MS2 (DIA) method for screening other present unknown pesticides or their transformation product. We have identified many compounds at Schymanski identification uncertainty 2 or 3.

Consequently, we have purchased reference standards for the most interesting ones and developed a targeted method. We aimed to investigate how valid the abundance of unknowns expressed like peak area is for ranking and prioritizing pesticide selection for targeted management on the local scale, i.e., if using peak area only can be applied for local pesticide management. The study was conducted within the project of the Ministry of Agriculture of the Czech Republic, National Agency for Agricultural Research (project no. QK23020018).

### **3.15.P-Mo259 Spatial Heterogeneity of Soil Properties Shapes Residual Pesticide Concentrations on the Meter-Scale**

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Conventional agricultural management often includes field-wide, mostly homogenous application of pesticides without considering the spatial heterogeneity of soil properties or the degradation potential of microbial degrader communities. The environmental fate and soil residual concentrations of pesticides, however, vastly depend on the soil properties and environmental conditions. In an agricultural field (0.5 km<sup>2</sup>) we here determined the meter-scale spatial soil heterogeneity at 60 different locations at two soil depths and correlated the data to residual concentrations of pesticides (and their transformation products) that had been applied in the last ten years. While there were small differences between the two soil depths, we found a significant spatial soil heterogeneity of soil texture (loamy sand to clay soils; 2.0-87% sand content), pH (5.6-7.3), water holding capacity (25-67%), total nitrogen (TN; 8-24%), soil organic carbon (SOC; 0.8-2.5%), and 16S rRNA (3-13 \* 10<sup>9</sup> copies g<sup>-1</sup><sub>dw</sub>) and ITS (9-114 \* 10<sup>7</sup> copies g<sup>-1</sup><sub>dw</sub>) gene copy number as proxies for bacterial and fungal biomass. 34 herbicides, 11 fungicides, and 5 insecticides were detected in our samples with the herbicide chlortoluron being the most abundant compound at concentrations of up to 58.1 ng g<sup>-1</sup> in clay loam soils. 21 of the pesticides were detected in all soil samples. Total pesticide concentrations exhibited positive correlation with TN, SOC, fungal biomass, and the ratio of ITS to 16S rRNA gene abundance as indicator of soil microbial community composition, while they negatively correlated with the sand content, the WHC. By contrast, lower concentrations of transformation products were detected in soil with lower ITS to 16S rRNA gene abundance ratios, lower sand content and higher water holding capacity, resp. Our data demonstrate high meter-scale spatial variability with concomitant effects on residual pesticide loads should be considered in future precision farming applications. Our ongoing work relates intra field-specific heterogeneity to microbial pesticide degradation and determines its potential effects on toxic endpoints by bio-analytical tools.



### 3.15.P-Mo260 Pesticides in Agricultural Soils: Major Findings From Various Monitoring Campaigns in Switzerland

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Synthetic pesticides are widely applied in modern agriculture, where they are used against diseases, pests, and weeds to secure crop yield and quality. However, their intensive application has led to widespread contamination of the environment, including soils. Due to their inherent toxicity, they might pose a risk to soil health by causing harm to non-target organisms and disrupting ecosystem services in both agricultural and other exposed soils. Following the Swiss National Action Plan on the reduction of pesticide risks, Agroscope has conducted several soil monitoring studies that are briefly presented here. All of them resort to different multi-residue trace analytical approaches to simultaneously quantify up to about 150 modern pesticides by either accelerated solvent, or Quick, Easy, Cheap, Efficient, Rugged, Safe (QuEChERS) extraction, followed by separation and detection with liquid chromatography-triple quadrupole mass spectrometry. While partly still in progress, our investigations led to the following major findings this far: Multiple pesticides are commonly present in soils, with individual concentrations in agricultural soils often reaching up to a few tens of µg/kg. Pesticide occurrence and concentrations in agricultural soils primarily depend on land use, land use history and cultivated crops. Pesticides can prevail much longer than predicted by their half-lives, and were found in soils even decades after conversion from conventional to organic farming. Corresponding residual fractions can be in the order of a few percent of the originally applied amounts. We further found negative associations of pesticide residues with the abundance of beneficial soil life, underpinning their potential risk to the fertility of agricultural soils. Traces of pesticides are also detected in soils to which they were never applied, indicating contamination, e.g., via spray drift or atmospheric deposition. These results confirm the general notion of both scientists and legislators that prospective risk assessments (RA; as executed during registration and use authorization) should be confirmed and adjusted by retrospective RA (e.g., by environmental monitoring studies of currently used compounds) to jointly lead to an overall reduced environmental risk of pesticides.

### 3.15.P-Mo261 Current Use Pesticides in Spatial and Temporal Distribution in Soil and Vegetation of Agricultural Sites

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In recent decades, awareness of the potential impact of pesticides on the environment has increased. Pesticides are widely used in agriculture to control pests and diseases and to increase crop yields. However, they can also have negative impacts on the environment and are also discussed in the context of insect decline. Insects developing in soil and feeding on vegetation are exposed to pesticides. To gain a better understanding of the presence and seasonal variations of pesticide residues in agricultural landscapes, a comprehensive study was conducted to better understand realistic exposure of insects.

The main objective of this study was to record current use pesticide residues in fields and meadows over a period of one year to cover the seasonal change of occurring pesticides. For this purpose, monthly soil and vegetation samples were taken from different agricultural fields (viticulture, vegetable fields, arable crops, three fields each) in the crop and at three points of increasing distance (1 m, 5 m, 20 m from the field) in adjacent meadows. The analyses for residues of 94 different pesticides were carried out with a high-performance liquid chromatograph coupled with an electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) to ensure accurate qualification and quantification of residues. To our knowledge this is the first temporal pesticide distribution dataset for soil and vegetation.

Our unique dataset shows that over the course of a year, different current use pesticides (CUP) were detected in sampled soil and vegetation from agricultural fields, but also from untreated meadows in their surroundings. We detected a variety of pesticides in different concentrations and mixtures with up to 28 pesticide residues in soil (Ø 10.3 CUPs per soil sample) and 25 in vegetation (Ø 7.24 CUPs per vegetation sample), with residues of some substances (fluopyram, boscalid) being recorded in almost all samples. Seasonal fluctuations were observed in vegetation samples, while almost no changes were observed in the topsoil samples over the course of the year.

The available data provide essential insights into the long-term fate of pesticides in the environment and provide realistic exposure data for insects in agricultural landscapes. Our results can help to develop targeted measures to reduce pesticide exposure and a protective environmental risk assessment.

### 3.15.P-Mo262 Contaminated Landscapes – Current Use Pesticides Residue Measurement in the Upper Rhine Valley

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Pesticides are applied on crops and would optimally be restricted to agricultural fields. Yet they have been detected in non-target areas including nature conservation areas, that serve as refuges for protected plant and animal species and are therefore of particular concern. This research aims to examine the landscape scale distribution of current-use pesticides in the Upper Rhine Valley in Germany, a region characterized by intensive agricultural practices in the valley and remote areas in the Palatinate Forest and Black Forest. In 2022, vegetation, soil, and water samples were collected at 78 non-target, off-crop sites

in 6 systematic 30 km long transects with west/east orientation during the pesticide application season and subjected to a comprehensive extraction and analysis that included 94 different pesticides. In total, 68 different pesticides (29 fungicides, 22 herbicides and 17 insecticides) were detected in the samples (n=186). Residues were detected in 97% of all vegetation (n=78), 97% of all soil samples (n=78) and 87% of all water samples (n=30). In total, 144 unique mixtures with  $\geq 2$  components were recorded. Samples in off-crop habitats in agricultural landscapes (valley) showed 90 unique mixtures, whereas 57 unique mixtures were detected in mountain regions. A higher mean number of CUP detections was recorded in the valley (mean = 10.1) than in the mountain region (mean = 3.2) across all sample types. Applying the pesticide detections and site-specific parameters in a linear model, a prediction map was generated to illustrate the extent of pesticide exposure across the entire landscape of the Upper Rhine Valley on 50 by 240 km. To our knowledge this is the largest scale exposure model available for a Central European landscape. Specific spatial weightings for land use types were identified. The results indicate a widespread presence of pesticides that extends beyond areas where pesticides are applied and reaches remote areas such as the Black Forest National Park. Of particular concern is the significant number of pesticides predicted in small nature conservation areas, surrounded by agricultural areas. This study indicates the need for a re-evaluation of the distribution potential of frequently detected pesticides and the development and implementation of a mixture risk assessment approach for terrestrial organisms in current pesticide regulation schemes.

### 3.15.P-Mo263 Occurrence and Persistence of Pesticides in Agricultural Soils from Different Viticulture Areas

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Modern agriculture uses large amounts of pesticides (i.e., fungicides, insecticides and herbicides) in order to increase the productivity and profitability of crops. The Sustainable Development Goals require combine the access to cost-affordable and safe food commodities with the preservation of the agricultural soil fertility and its biodiversity, preventing accumulation of these compounds in soil and/or their migration to other environmental compartments, including wildlife animals, live-stock and aquatic media. Moreover, policies derived from the Green Deal aim a 50% reduction in the use of agricultural pesticides by year 2030.

Viticulture is one of the agricultural practices using the highest ratios of fungicide per unit of surface. As a permanent crop, vineyards have been sprayed with this group of compounds for decades, which might lead to accumulation of those compounds with degradation periods longer than the annual application and rest cycles of vines. In addition to fungicides, vineyards are also treated with insecticides to control different pests (i.e., *Lobesia botrana*) and herbicides. The latter ones are particularly used in those plots where mechanization results complex due to limited distances between the rows of vines, and/or the slope of vineyards in hilly areas.

Herein, a target liquid chromatography triple quadrupole mass spectrometry (LC-QqQ-MS) procedure was developed to assess the occurrence and persistence of 80 pesticides, including mainly fungicides, but also insecticides and herbicides, in composite samples of topsoils (0-15 cm layer) collected in the geographical areas of La Rioja and Galicia. Samples corresponded to different sub-zones, or Qualified Designation of Origin, aiming to obtain a plot of soil samples existing in areas affected by different kinds of pests. Persistence of pesticides in soils was assessed through three consecutive sampling seasons. A range of currently approved and already expired pesticides were identified in the analysed samples. Among them, several azolic fungicides, boscalid, fluopicolide and the herbicide diflufenican were identified as persistent compounds, with dissipation periods longer than the rest period of vines.

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### 3.15.P-Mo264 Fungicides Residues in Surface and Groundwater Samples from High Pressure Viticulture Areas

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Controlling the pollution of continental waters by anthropogenic compounds is a challenging issue in a context of sustainable development. In rural areas, agriculture activities use significant amounts of pesticides, with a variable persistence and/or mobility in the different environmental compartments. As example, neonicotinoids are known to be prone to fast photochemical degradation; however, they are stable in soil and highly soluble in water; thus, they might migrate into groundwater. Furthermore, relatively non-polar compounds might reach surface water environments associated to soil particles transported with run-off water during rainfall events.

Viticulture uses large amounts of fungicides and insecticides sprayed directly on the canopy of vines. A fraction of the applied compounds reaches the vineyard soil during application. Washing from vine leaves also contributes to their transport to the topsoil layer. Thereafter, these pesticides might migrate through the soil profile reaching groundwater, or being transported to surface water bodies, both dissolved and associated to soil particles. Erosion of topsoil in vineyards from hilly areas might be a relevant input source of pesticides in non-permanent streams and rivers. Another potential source of pesticides in ground- and surface water from vineyard areas is the misuse, aerial diffusion during application and/or disposal of sprayers' washing waters.

The aim of this work was to develop a sensitive, automated methodology for the determination of pesticide residues in surface and groundwater samples, obtained from springs and wells, located at different vineyards in Galicia and La Rioja (Northern Spain). Samples were concentrated using solid-phase extraction on-line combined with liquid chromatography coupled to mass spectrometry detection. The final procedure achieved limits of quantification in the range of low ng/L with a linear response range up to 2000 ng/L. Analysis of surface and groundwater samples taken at the beginning, middle and end of the growing season of vines reflected relevant contamination problems mostly in groundwater.

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### **3.15.P-Mo265 Widespread but Different – Synthetic Pesticides Show Strong Spatiotemporal Dynamics While Copper Pollution Is Generally High Across Swiss Vineyards**

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The intensive use of plant production products (PPP) has led to a widespread accumulation of those substances in agricultural soils, and we are only at the beginning of understanding their ecological consequences. Vineyard soils are especially exposed due to the large amounts, broad range, and repetitive use of PPP. A comprehensive study on exposure of vineyard soils to PPP should therefore cover a large number of synthetic pesticides and also consider copper-based fungicides that play an integral role in vine protection, especially in organic farming. Further, the strong within-field heterogeneity of vineyard soils should be taken into account that originates from differences in vine row and inter-row management and low turbation of the soil layers.

To represent Swiss viticulture, 62 conventional and organic vineyards in three major winegrowing regions were analyzed. Samples were taken separately in vine row and inter-row and split into top and bottom soil. Soil samples were analyzed with a newly developed method covering 146 synthetic pesticides and transformation products. Copper concentrations were measured with two methods to get a proxy for total copper and bioavailable copper.

For synthetic pesticides, up to 60 substances per vineyard were detected and strong differences between management systems were found. There was a clear decrease in synthetic pesticides with increasing years of organic farming, but after more than 20 years up to 32 pesticides could still be found. The clear regional differences found might be explained by climate and soil properties. As hypothesized, soils displayed a high within-field heterogeneity and the distinctness of those within-field differences depended on management type and region. In contrast, copper pollution was not affected by management type or region and was generally high. Organic vineyards did not display higher copper concentrations than conventional despite the limited availability of substitutes. We argue that copper pollution is mainly explained by historic applications.

Our study confirmed a generally high pollution of Swiss vineyard soils with PPP. Strong contrasts were found between copper with an even distribution and synthetic pesticides which responded strongly to management, regional, and within-field differences. Further, we stress that future studies on vineyard soil pollution should consider within-field heterogeneity in their sampling strategy.

### **3.15.P-Mo266 Point Source Contamination via Sprayer Cleaning Water Dispersal: A Northern Italian Vineyard Area Case Study to increase local farmers' awareness**

*Nicoleta Suci*<sup>1</sup>, *Ettore Capri*<sup>2</sup>, *Anastasia Lomadze*<sup>3</sup>, *Terenzio Bertuzzi*<sup>4</sup>, *Gian Maria Beone Prof*<sup>4</sup>, *Emanuela Delpero*<sup>5</sup>, *Maura Calliera*<sup>6</sup>, *Alessandro Varotto*<sup>5</sup> and *Stefano Bergaglio*<sup>5</sup>, (1)Università Cattolica del Sacro Cuore, Italy, (2)Università Cattolica del Sacro Cuore, Italy, (3)Catholic University of the Sacred Heart (UNICATT), Italy, (4)Università Cattolica del Sacro Cuore, (5)Anadiag srl, (6)European Observatory on sustainable agriculture (OPERA), Università Cattolica del Sacro Cuore, Italy Point source contamination by pesticides is a problem of increasing concern and represents an important threat due to the potential adverse effects of these substances on the non-target organisms of soil and natural water resources. Point source contamination could occur as a result of spillages, leakages, and mismanagement of pesticide-contaminated water produced during in-farm and post-farm activities. In order to evaluate the relevance of the impact of wastewater resulting from the cleaning operations of the fitosanitary application equipment and understand the farmers' awareness level regarding the environmental impact of in farm point source contamination, a participatory monitoring campaign was conducted across five viticultural farms located in Tortona hills. The content of six organic plant protection products (PPPs) and of Cu were analyzed

in 52 samples of external and internal washing water, collected in the period May-August 2022 and 2023 in five farms (3 conventional and 2 organic). Considering the quantity of water used for the cleaning operations, the concentrations found in the washing water, the quantity of the active ingredient used for each treatment, and the vineyard area treated, it was possible to quantify the percentage of the active ingredient dispersed with each washing. The estimated percentage of the active ingredient dispersed with washing water ranged from 0.001% to 53.90% for organic active ingredients, while from 0.01% to 0.4% for metallic Cu. The results have been shared with the participating farms with the aim to increase their awareness about the necessity of proper management of the wastewater generated after cleaning spraying equipment.

### **3.15.P-Mo267 Challenges Conducting Higher Tier Groundwater Monitoring Studies in France**

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Targeted groundwater monitoring studies represent a higher-tier approach in the regulatory evaluation of plant protection products. Recent scientific recommendations have outlined and evaluated their design and conduct. The key objective is to align with defined exposure assessment goals (ExAGs), focusing on measured environmental concentrations in shallow groundwater within aquifers susceptible to leaching in the designated product application area. However, ExAGs are seldom defined, as exemplified by the current situation in France.

While comprehensive national guidance is lacking, the French regulatory agency (ANSES) impose specific requirements. French studies have to focus on catchments linked to (1) active water supply wells, and/or (2) wells in the French Water Database (ADES) with prior analyte detection. Additionally, attention is given to (3) wells in vulnerable areas using the product, encompassing diverse terrains like karst landscapes, fractured rock formations, and alluvial aquifers. Specific considerations, including soil pH, are incorporated into this targeted methodology with the intention to derive proper risk mitigation measures based upon environmental descriptors of most vulnerable areas.

These requirements pose a number of challenges in finding suitable sites: (1) assuring well quality; (2) assessing shallow unconfined groundwater with limited seasonal fluctuation; (3) securing multi-year access with water suppliers; (4) having a manageable catchment size to enable characterisation of soils, hydrogeology, crops and pesticide usage history; (5) engaging farmers for data sharing; (6) selecting areas where the crop is frequently grown despite rotation; and (7) ensuring active substance usage across the catchment at the desired rate to limit false negatives and support the GAP being applied for. Additionally, the requirements support ExAGs that differ from those supported by edge-of-field sites. This results in a mixed European Union monitoring data package where French data may be considered only supportive in other Member States and vice-versa. This poster presentation aims to elucidate the complexities of this monitoring approach in France, providing examples and exploring potential implications for the results.

### **3.15.P-Mo268 From Field to Catchment: Comparing the Impact of Groundwater Exposure Assessment Goals for a Case Study in France**

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The regulatory framework in the European Union describes a tiered approach to assess the environmental impact of plant protection products on groundwater. In lower tiers, mechanistic modeling approaches and pre-defined scenarios are used to predict groundwater exposure. Predictions are refined by a spatially distributed assessment in Tier 3b, which simulates combinations of soils and weather conditions occurring within the evaluated area of interest. Currently, there is no consensus at EU or member state level on how to design Tier 3b assessments which are universally accepted and regulatory binding.

Current scientific recommendations on Tier 3b are closely aligned with FOCUS guidance, such as (a) assuming a product will be used in the highest frequency and rate that is registered or being applied for, (b) disregarding dissipation processes below one metre soil depth, and (c) calculating the exposure assessment goal (ExAG) from leachate concentrations at one metre depth. Spatial exposure assessments akin to Tier 3b are recommended to support the site selection in Tier 4 groundwater monitoring studies. However, using Tier 3b results to characterise Tier 4 monitoring sites may require consideration of additional factors to increase realism if ExAGs are defined on a (sub)catchment basis.

A groundwater monitoring study was conducted in France in compliance with national regulations and scientific guidelines. Diverging from the conventional approach, the study focused on catchments of water supply wells rather than the edge-of-field design. Results of a Tier 3b assessment were used to derive national exposure estimates and were refined to account for realistic product use and cropping practices to reflect agronomic realities in France. In addition, the ExAG was adjusted in line with French requirements to represent the risk to groundwater wells more accurately. The exposure risk of groundwater sampled from a well is defined by additional fate processes below one metre depth and aggregated substance exposure in its catchment, which are influenced by temporal and spatial factors which are not necessarily reflected in current scientific recommendations. This refinement contributed to a more comprehensive understanding of observed Tier 4 groundwater exposure and enabled the estimation of an inherent safety factor, establishing a link between the tiers.

### **3.15.P-Mo269 Contrasting dissipations of legacy and current pesticides from agricultural soil to drainage water during potato cultivation challenge traditional risk assessment models**

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Pesticides are widely applied to agricultural crops to control weeds, plant diseases, outbreaks of insects and other pests. Although designed to target specific pests only, the intensive pesticide use also poses a risk to beneficial organisms and inevitably results in the distribution of pesticides into different environmental compartments. Several models using pesticide persistence (DT<sub>50</sub>) and leaching potential (groundwater ubiquity score - GUS) are currently used to assess the environmental pesticide risk. In this study, we simultaneously quantified 18 pesticides in soil and drainage water during a conventionally grown potato culture at high temporal resolution. The measured pesticide concentrations were then fitted to the above models. Overall dissipations of all freshly applied compounds in soil were in line with published DT<sub>50</sub> field values and their occurrences in drainage water were generally consistent with GUS model, documenting that the fractions of pesticides exported via drainage water were proportional to their leachability. In contrast, concentrations of the legacy pesticide atrazine (last application in 2009) and one of its transformation products (atrazine-2-hydroxy) were stable during the entire sampling campaign. Moreover, atrazine concentrations in drainage water were substantially diluted during peak discharge, whereas those of freshly applied pesticides were at maximum, concomitantly with the first drainage water discharge event after the respective spray application (first flush effect). In conclusion, the applied risk assessment models were capable of predicting environmental concentrations and dissipation of pesticides at the short and medium time scale of a few half-lives, but felt short of capturing long term trace residues.

### **3.15.P-Mo270 Assessing Plant Protection Product and Transformation Product Contamination in the Soils Surrounding Lentic Small Water Bodies: A High-Resolution Study in Northern Germany**

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Lentic small waterbodies (LSWB) are widespread worldwide and play a diverse ecohydrological role that is crucial for biodiversity in rural areas. Due to their unique size and location, LSBW exhibit complex interactions with their surroundings that are not present in other water bodies. Despite their importance, there is a limited database on the pollution of LSBW by plant protection products (PPP) and their transformation products (TP), with transport pathways and pollution sources poorly understood.

This study addresses the exposure of PPP and TP in soils in the vicinity of two LSBW in northern Germany and highlights the importance of long-term storage and as a potential source of contamination for LSBW. From September 2020 to April 2022, monthly composite samples were taken from the inflow and outflow areas of the LSBW at four depths each. The soil samples were extracted with CaCl<sub>2</sub> and MeOH solutions to analyze both bioavailable and potentially available compounds. The extracted samples were quantified for 26 PPP/TP by LCMS analysis. In addition, chemical and physical soil parameters were determined, and soil moisture was measured continuously at three depths at both LSBW.

The results show the presence of PPP/TP in all samples and demonstrate the degradation, transformation, and leaching of various applied PPP over several months, including herbicides such as flufenacet and metazachlor. The degradation rates of the applied PPP were determined. Non-applied substances such as the fungicide bixaphen were consistently found, reflecting the persistent long-term storage of PPP/TP in the soil. The MeOH extraction showed the contamination of the soil by PPP/TP with the highest results, while the aqueous extraction revealed the availability and easy solubility of the particularly mobile PPP/TP, whether applied or not, indicating their potential translocation to shallow groundwater. The high-resolution measurement campaign improves our understanding of the dynamics of PPP/TP in soils adjacent to LSBW. In the next steps, the findings will be compared with further results from groundwater and LSBW examinations to better comprehend the soil as a potential pollutant source for the LSBW.

### **3.15.P-Mo271 Glyphosate and Aminomethylphosphonic Acid (AMPA) in Surface Water from the Tagus River basin**

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The present study investigated the presence of glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) in surface water samples (n = 89) collected from the Tagus River watershed during 2020, 2021 and 2022. The analytical method included derivatization with 9-fluorenylmethoxycarbonyl chloride (FMOC-CL), solid-phase extraction, high performance liquid chromatography tandem mass spectrometry (HPLC-TqMS/MS) and isotope dilution for quantification. Limits of quantifications meeting SANTE/2020/12830 and SANTE/11312/2021 criteria were set at 5 ng/L for both analytes.

High quantification frequencies were obtained for both glyphosate (89%) and AMPA (96%). Currently, there is no environmental quality standard (EQS) for glyphosate or AMPA at the EU level. However, glyphosate concentrations ranging from N.D. to 641 ng/L offered annual average (AA) concentrations (120, 115 and 104 ng/L for 2020, 2021 and 2022) well below the threshold included in the proposal for amending the Water Framework Directive (86.7 µg/L AA-EQS and 398.6 µg/L maximum allowable concentration MAC-EQS) in inland surface waters. Nevertheless, glyphosate annual average concentrations are slightly higher than the limit of 0.1 µg/L (AA-EQS) proposed for freshwater used for the abstraction and preparation of drinking water. No seasonal or temporal (2020-2021-2022) tendencies (p>0.05: Kruskal-Wallis test) were found. However, samples collected near urban areas presented higher (p<0.05: Mann Whitney U Test) glyphosate and AMPA

concentrations compared to those obtained from agricultural/ rural zones, suggesting that the presence of these pollutants in the Tagus River basin could be mainly related to nonagricultural uses. Besides, samples with high urban influence, revealed lower AMPA/Glyphosate ratios suggesting a fresh glyphosate input. Similar behavior was also observed for 2 agricultural areas where the use of glyphosate in irrigated crops was tentatively identified as the major source. Glyphosate risk assessment conducted for aquatic organisms revealed a negligible ( $RQ < 0.01$ ) or low risk ( $0.01 < RQ < 0.1$ ) at median (P50) and worst case (max) scenarios. However, AMPA levels may pose a medium risk ( $0.1 < RQ < 0.1$ ) in 8 of the 13 sampling points even at P50.

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### **3.15.P-Mo272 Glyphosate and Aminomethylphosphonic Acid (AMPA) in Spanish Indoor Dust**

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Glyphosate is the most employed herbicide in Europe. However, it is also a source of controversy since the World Health Organization's cancer agency concluded in 2015 that it was probably carcinogenic to humans. Recently, the European Union Commission adopted the Implementing Regulation to renew, for 10 years, the approval of glyphosate. Nevertheless, the last EFSA's peer review report on glyphosate (July 2023) identified data gaps that should be addressed. For this reason, the purpose of this work was to investigate the occurrence of glyphosate and aminomethylphosphonic acid (AMPA), its main metabolite, in Spanish indoor environments (homes, offices and schools). The validated analytical method (SANTE 2020/12830 and SANTE 12682/2019) including derivatization with 9-fluorenylmethoxycarbonyl chloride (FMOC-CL) and UHPLC-MS/MS analysis, was applied to 50 settled (<0.5 m) and suspended (>0.5 m) dust samples. Both homes, offices and schools presented glyphosate and AMPA levels above limits of quantification (50 and 100 ng/g; LOQs for glyphosate and AMPA, respectively). Dust concentrations range between (0.05: Mann-Whitney test) were obtained between suspended and settled dust in terms of glyphosate and AMPA levels, nor between indoor environments ( $p > 0.05$ : Kruskal-Wallis test). Besides, no correlation was found between both analytes, and AMPA/glyphosate ratios range from 6 to 35, reflecting high dispersion in the data obtained. Risk assessment conducted for dust ingestion revealed no risk since Estimated Daily Intakes (EDIs) for infants (<6 months; 0.27 ug/Kg bw/ per day) and adults (0.01 ug/Kg bw per day) at worst case scenario (glyphosate and AMPA maximum concentrations) were well below the acceptable daily intake (ADI) of 0.5 mg/kg bw per day proposed by EFSA.

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### **3.15.P-Mo273 The Glyphosate Dilemma: The Tug-Of-War Between Food Security And Environmental Health**

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Agriculture is vital for South Africa's economy. Glyphosate-based herbicides (GBHs) are common, as crops are genetically modified for glyphosate (GLY) resistance. Despite widespread use, limited data exists on GLY and its metabolite, aminomethylphosphonic acid (AMPA), in South Africa's environment. GBH applications for maize, soybeans, wheat, and sunflower represent 80% of total use (15 484 tonnes). By visualising these four crops with geographic information systems, one of the main maize-growing provinces, the Free State, had the highest applications, with 1 753 tonnes in 2017 and 1 065 tonnes in 2019 according to an international market research company. This was mostly located along the Vaal River, which is the hardest working river in the country. Maize dominated GBH use at 74% in 2017 and 61% in 2019, with soybean applications increasing from 19% to 28%. About 2–2.5% of GBH use has non-agricultural application. South Africa, sub-Saharan Africa's largest pesticide consumer, saw GBH use double from 3 721 tonnes in 2009 to 7 977 tonnes in 2017, slightly decreasing to 7 507 tonnes in 2019. Concerns about non-target biota risks and human health effects led to GBH bans in some countries, but not in South Africa, despite the local cancer association labelling glyphosate as a group 2A carcinogen. Using data from 2017 and 2019, we chose sampling locations in a rainfed area, an irrigation scheme, and some non-agricultural sites. Analysis by ultra high-pressure liquid chromatography coupled to mass spectrometry of samples collected after spraying resulted in no quantifiable concentrations of GLY or AMPA. The limit of detection (LOD) for GLY and AMPA in water samples was 300 and 200 ng/L, respectively. In sediment, the LOD for GLY and AMPA were both 500 ng/L (0.042 ng/g when back calculated for the soil extracted). These results sharply contrasted with the expectation. One possible explanation is that other than GBH herbicides were used. Confirmation of reportedly-used herbicides is absent due to unreliable communication of pesticide-use by the farming community. The outcomes of this study emphasizes the need for establishing regular environmental monitoring of GLY and AMPA covering a greater surface area of the country to realistically assess the use of GBH and therefor realistic risk assessment.

### **3.15.P-Mo274 The impact of rice cultivation systems in a Mediterranean wetland: Evaluation of the occurrence, behaviour and fate of pesticides and their transformation products**

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L'Albufera Natural Park, in Valencia (Spain), is a Mediterranean wetland mainly devoted to rice cultivation. Therefore, it is

crucial to assess the impact of pesticides on the environmental compartments (water, sediments and biota) affected by agricultural activities. Moreover, the accumulation of organic pollutants has considerably degraded these areas, which may adversely affect human health.

The objective of the study was to evaluate pesticides accumulating in rice growing areas including identification of major metabolites. The study included the identification of the main transformation products of pesticides. The sampling campaign was performed from May to September 2021 during the rice harvest period. Samples were collected every week to compare the emissions of pesticides. Four sampling cases were considered: a local spring (called Ullal), one organic production system and two other sampling points with traditional crop production systems (Sollana and Alfafar) to obtain information regarding the accumulation, transport, and degradation of pesticides in water and sediments. Pesticide application history in this field has been recorded.

Pesticides were extracted from water samples by solid phase extraction and sediments by the citrated buffered QuEChERS. Pesticides were determined by LC-HRMS in an Orbitrap Exploris 120 mass spectrometer. Pesticides and their transformation products were determined using wide target screening against a positive list of compounds and non-target screening by applying ddMS2 of the four more intense ions in each cycle. The target analysis was focused on 10 pesticides used during rice cultivation (azoxystrobin, cyhalofop-butyl, 2-methyl-4-chlorophenoxyacetic acid, penoxulam, propanil, difenoconazole, bentazone, cyhalofop acid, imazamox and acetamidrid) as well as their transformation products.

The results revealed the presence of pesticides in varying amounts in water and sediments, even within organic parcels employing eco-friendly planting strategies. The occurrence of these pesticides is directly associated with irrigation water that, in many cases, goes from one field to another and the proximity to crops from conventional production systems. However, Ullal showed very low pesticide levels in comparison to conventional systems.

### **3.15.P-Mo275 Pesticides Screening on Surface Water and Soil along the Mekong River in Cambodia**

*Putheary Ngin, Chemistry, Umea University, Sweden*

The widespread use of pesticides has raised serious concerns about environmental contamination, particularly with regard to aquatic and soil ecosystems <sup>(1)</sup> and the screening of pesticides in those environmental matrices in Cambodia has never been studied, published studies have focused on vegetables or rice <sup>(2&3)</sup>. The study assessed pesticide residues in surface water and soil across four Mekong River provinces in Cambodia during dry and rainy seasons, analyzing 276 samples for 64 pesticides. A semi-structured interview was conducted to identify pesticide use by local farmers and an ecological risk assessment of the detected pesticide residues in surface water was evaluated using risk quotients (RQs). A total of 56 pesticides were found in surface water, and 43 in soil, reaching maximum concentrations of 2,600 ng/L and 1,100 ng/g dry weight, respectively. Tebufenozide and bromophos-ethyl showed the highest concentrations in surface-water and soil samples, present in nearly all samples. The semi-structured interview showed that the farmers tended to apply more insecticides, especially when they observed certain pests in their fields. Inappropriate pesticide use, including the timing, frequency, concentration, and type of products used, is widespread. Risk assessment indicated high ecological risk, especially for bromophos-ethyl, dichlorvos, and iprobenfos. Overall, this work highlights the occurrence of pesticide residues in surface water and soil along the Mekong River in Cambodia and emphasizes the urgent need for enhanced monitoring and regulation of pesticide use in the region.

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### **3.15.P-Mo276 Exposure and Combined Risk of Pesticide Mixtures in Wetlands of the Great Barrier Reef Catchment Area, Australia**

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Wetlands of the Great Barrier Reef Catchment Area (GBRCA) provide a range of ecosystem services supporting the health and resilience of the GBR ecosystem, many of which serve to protect the GBR from land-based effects including acting as pollutant traps for pesticide runoff from local agriculture. However, knowledge of the occurrence of pesticides in GBRCA wetlands is limited, leaving them vulnerable to excessive loading of pesticides resulting species decline, loss of value, reduced function of ecosystem services.

The aim of this work was to investigate the combined risk from the occurrence of pesticides in wetlands of the GBRCA. Located within the GBRCA, the Herbert River Floodplain is designated as an area of Nationally Important Wetlands, though



surrounding agricultural land use puts the health and function of these wetlands at risk. Five freshwater wetlands of the Herbert River Floodplain were monitored using a combination of grab (collected monthly) and passive sampling (approx. 1-month deployments) techniques between February 2021 and March 2023. Samples were analysed for 55 pesticides by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS).

Thirty-six pesticides were detected across five wetlands, 8 of which exceeded regulatory guidelines at least once. The combined risk of 19 commonly detected pesticides was assessed using a novel Pesticide Risk Metric (PRM) model, calculated as the acute (grab samples) and chronic (passive samples) percentage of species affected. Wetlands are afforded either a 95% or 99% species protection goal based on the surrounding land use and conservation status. Forrest Beach wetland – located within a conservation zone, was the only monitored wetland to continuously meet its species protection goal of 99% throughout the study period. The greatest number of PRM values exceeding the site-specific species protection goal was at Legges Road wetland – afforded 95% species protection, where 9 daily and 12 monthly PRM values were >5% species affected. Seasonal rainfall is known to be a driver of pesticide occurrence and risk in surface waters of the GBRCAs, so wet/dry season was investigated as a driver of risk in wetlands. The monthly average (chronic) risk was found to be increased in two wetlands during the wet season, though no increase in daily (acute) risk was detected in the season at any site. These findings indicate that wetlands behave differently to rivers and creeks in the GBRCAs.

### **3.15.P-Mo277 Estimation of glyphosate and AMPA mass loads from twenty-two Wastewater Treatment Plants across Australia**

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Glyphosate is the most frequently used herbicide globally yet data on its prevalence in Australia is scarce. This study aimed to investigate glyphosate concentrations and release in Australian wastewaters and wastewater treatment plants (WWTPs). Glyphosate and its main biodegradation product, aminomethylphosphonic acid (AMPA) highly polar nature has historically made detection and investigation challenging. After concentrations are determined, mass loads were then calculated for effluent WWTPs. Sub-aims included assessing the spatial and temporal trends of glyphosate and AMPA in WWTPs from 2009-2021, determining the removal efficiency of WWTPs, and assessing the stability of glyphosate in WW samples over a year. The study found that glyphosate was present in the influent of all sampled WWTPs at concentrations of  $22 \pm 76 \mu\text{g/L}$ . Glyphosate was detected in 18 WWTP sites sampled in both pools 1 and 2, with concentrations in effluent ranging from <LOD -  $11 \mu\text{g/L}$ , the highest concentration being at site 6, with an average of  $2 \pm 3 \mu\text{g/L}$ . Glyphosate removal efficiency varied depending on the treatment process used, but were generally effective except in primary WWTPs, which showed a negative removal efficiency. The mass loads of glyphosate in effluent were calculated at 572.3mg per day per 1000 people. Temporal trends for glyphosate show an increase in use from 2009-2021. This study is the first-time characterisation of glyphosate and AMPA in Australian WWTPs. Given the toxicity claims to humans and species of glyphosate in the environment, determining its presence in WWTPs and estimating its mass loads can help inform regulatory bodies on managing exposure to glyphosate.

### **3.15.P-Mo278 Down-The-Drain Pathways for Fipronil and Imidacloprid Applied as Spot-on Parasiticides to Dogs - Estimating Aquatic Pollution**

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Fipronil and imidacloprid have been widely detected in UK surface waters in recent years, often at concentrations that ecotoxicological studies have shown can harm aquatic life. Down-the-drain (DTD) passage of pet flea and tick treatments are being implicated as an important source, with many of the UK's 22 million cats and dogs receiving routine, year-round preventative doses containing these parasiticides. The UK Water Industry's 3<sup>rd</sup> Chemical Investigation Programme (UKWIR CIP3) has confirmed wastewater as a major entry pathway for these chemicals into surface waters, but the routes by which they enter the wastewater system remain unclear. We addressed this knowledge gap by conducting the first quantification of DTD emissions from 98 dogs treated with spot-on ectoparasiticides containing fipronil or imidacloprid, through bathing, bed washing and washing of owners' hands. Both chemicals were detected in 100% of washoff samples, with bathing accounting for the largest emissions per event (up to 16.8% of applied imidacloprid and 24.5% of applied fipronil). Modelled to account for the frequency of emitting activities, owner handwashing was identified as the largest source of DTD emissions from the population overall, with handwash emissions occurring for at least 28 days following product application and an estimated 4.9% of imidacloprid and 3.1% of fipronil applied in dog spot-ons passing down-the-drain via this route. The normalised daily per capita emissions for all routes combined were  $8.7 \mu\text{g/person/day}$  for imidacloprid and  $2.1 \mu\text{g/person/day}$  for fipronil, equivalent to 20-40% of the daily per capita load in wastewater, as estimated from UKWIR CIP3 data. Within the current international regulatory framework adhered to by the UK, the environmental exposure of veterinary medicines intended for use in small companion animals is assumed to be low, and DTD pathways are not considered. We recommend a systematic review of regulations and practices to address this overlooked pollution pathway.

### **3.15.P-Mo279 Occurrence of pesticides in fish: a comprehensive monitoring study across Europe**

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It is widely known that the intensive and widespread use of pesticides can pose risk to environmental and human health. Pesticides applied in agricultural farms to safeguard crops can reach the nearby water bodies raising serious concerns on water quality and toxic effects on aquatic organisms. In the framework of the H2020 SPRINT project, the present study investigated the occurrence of pesticides, comprising 156 active substances and 34 metabolites, in the muscle and liver of 30 fish samples. Fish were collected during the 2021 growing season from water bodies of distinct typology (reservoirs, rivers, ditches, channels, lakes, streams, creeks and ponds) located in the vicinity of conventional/integrated and organic farms across 7 case studies in Europe (Croatia, Czech Republic, Denmark, the Netherlands, Portugal, Slovenia and Switzerland). Multiple pesticides residues were detected in fish (31 analytes: 13 in muscle and 24 in liver), of which 17 analytes were quantified (11 and 12 in muscle and liver, respectively). The insecticide folpet PHI exhibit the highest median concentration, quantified in all the muscle samples and in 12 liver samples). In the muscle, fluopicolide and terbuthylazine followed folpet PHI, while for the liver, the sequence included DDE, p,p', the fluopicolide, the diflufenican, and prosulfocarb. It is worth mentioning that the DDE, p,p' was the pesticide found in more samples of fish liver (19), and only in 1 sample for the fish muscle. Furthermore, the presence of the metabolite, DDE, p,p', derived from the banned DDT compound, remains a significant component of the environmental mixture of pesticides and therefore should be taken into consideration in further environmental risk assessments. Our results raise awareness of the potential risks posed by pesticides mixtures to aquatic ecosystems, which can ultimately be translated into human health impacts through the consumption of fish.

### **3.15.PC Measuring and Modelling the Environmental Fate and Exposure of Pesticides**

#### **3.15.P-Mo229 Particulate and Mineral Associated Organic Matter Contribution to Pesticide Sorption in Agricultural Soil**

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Intensive agricultural practices heavily rely on land exploitation and high inputs of pesticides to produce crop monocultures. Some applied compounds persist in soil, whereas the more mobile pesticides run off or leach through the soil profile and enter surface and groundwater sources. Soil organic matter content is recognized as one of the main factors determining the mobility and transformation fate of agrochemicals. However, to the authors' knowledge, the role of particulate (POM) and mineral-associated (MAOM) organic matter pools in determining pesticide fate and the implications for soil management practice choice is yet to be elucidated. This research aims to understand how the distribution of organic matter into the physical pools of POM and MAOM influences the sorption of pesticides in soil.

Using wet sieving, we fractionate the soil into the two physical fractions POM (> 53 µm) and MAOM (< 53 µm). After removing organic matter from POM and MAOM fractions, we obtain the rPOM and rMAOM, which serve as control treatments. We perform batch sorption tests to determine the efficiency of 9 pesticide removal from soil solution using separated and treated soil fractions. Our hypotheses are: 1) separated soil fractions with intact organic matter (POM, MAOM) will perform better in pesticide sorption than rPOM and rMAOM; 2) the nature of organic matter determines the extent of pesticide sorption efficiency, and MAOM will be more efficient than POM. The findings of this research can provide new insight into the effect of soil properties in reducing pesticide runoff, leaching, and subsequent environmental contamination and thus have important implications for choosing agricultural land-management practices (tillage, animal manure application, etc.).

#### **3.15.P-Mo230 On the search for alternative herbicides to treat Swiss railway tracks.**

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Weeds can have detrimental effects on the railway infrastructure. This mainly through the accumulation of humus, which can increase water retention and may damage both sleepers and rails, as well as the overall stability of the ground. Ultimately, these factors can result in track deformation, which directly influences the speed at which trains are able to travel.

In Switzerland, herbicides are predominantly utilized for weed control, although alternative methods such as mechanical and thermal techniques are also employed. Currently, glyphosate is the only herbicide used to remove weeds from railway tracks, which has proven to be relatively effective. However, uncertainties concerning the renewal of authorisations of the substance in Europe have prompted railway companies to explore alternative herbicides that may replace glyphosate. Due to the inherent permeability of railway tracks and therefore increased net infiltration, it is important to choose herbicides with a low risk of

leaching to groundwater. The characteristics of a suitable herbicide include thus a high sorption in the soil and/or rapid degradation (also for metabolites), as well as low application rates.

In order to help decision-makers to make informed decisions, we constructed ten lysimeters with railway materials from three locations, allowing us to study the leaching behaviour of herbicides in close-to-real conditions. Using liquid chromatography–mass spectrometry, we investigate the leaching potential of 12 alternative herbicides and their main metabolites. The selected group of alternative herbicides includes six auxin-mimics, three acetolactate synthase inhibitors, as well as three pigment-synthesis inhibitors.

Half a year after the first application of the substances in May 2023, we observe that leaching is influenced by both the lysimeter soil properties and the herbicides used. While some of the herbicides have already reached their maximum concentrations under all soil conditions, some have not been detected yet. These undetected herbicides may have degraded completely or may still elute later. The project will continue for at least 2 years (from the time of the first application) and will allow us to conclude on the leaching potential of the applied substances and their metabolites under realistic conditions. Moreover, the project will allow a direct comparison of all applied substances under identical weather conditions in 3 different soil types.

### **3.15.P-Mo240 Proposed revision of the aged-sorption guidance document in combination with field degradation studies in regulatory assessments**

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In the EU Aged Sorption Guidance the use of field DegT50 values in combination with aged sorption parameters is only possible to a very limited extent. If the leaching assessment for a plant protection product (PPP) is based on field DegT50 values only, it is not recommended by the current guidance to derive a DegT50<sub>equilibrium</sub> (rescaling of DegT50) for input in exposure models in combination with aged sorption parameters ( $f_{NE}$ ,  $k_{des}$ ) as described for laboratory DegT50 values. This virtually prevents the mitigating effect of aged sorption on leaching and limits the applicability of the aged sorption guidance in combination with field DegT50 to very few cases.

However, the guidance mentions that “Industry is preparing evidence for aged sorption in field studies and this option should replace the current recommendations as soon as appropriate guidance has been developed and tested”.

Therefore, additional field studies were evaluated to show the relevance of aged sorption in the field. The evaluation was mainly based on the comparison of observed concentration depth profiles versus FOCUS-PEARL simulations with and without consideration of aged sorption. Where possible, site-specific aged sorption parameters from laboratory studies with the respective field soils were used. The depth to which 95% of the residues are found was defined as metric [P5 metric] for comparison.

Proposed revisions to the current EU Aged Sorption Guidance fully allowing for the combination of field degradation and aged-sorption data are presented.

### **3.15.P-Mo241 Can Drainage Losses be Reduced by Optimizing Pesticide Application Dates?**

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Presently, in the EU risk assessment for plant protection products, there are no mitigation or refinement options available for drainage exposure apart from reducing the intended application rate and generic application time restrictions. One more flexible mitigation option could be to look in more detail at the application timing to omit the application at dates which lead to highest potential exposure via drainage. Recently published monitoring data (Tournebize et al., 2023) from the La Jaillière experimental site in France has revealed that drainage losses remarkably increased when soil moisture exceeded a certain threshold at the time of pesticide application, thereby increasing the pesticide concentrations in the receiving water bodies.

This study examines the role of soil moisture on the application day as a potential trigger in influencing exposure via drainage. Existing FOCUS and UK drainage scenarios were used to calculate a soil moisture trigger value in terms of the soil wetness index (SWI). Limiting the pesticide application to periods when the SWI is below this trigger value could reduce environmental safety concerns and enable safe uses with respect to drainage entries into water bodies. Three herbicides were chosen for this assessment. Step 3 MACRO runs were simulated with no interception and with additional hydrological outputs being switched on needed for calculating SWI on the day of application. Predicted environmental concentrations (PEC) in ditch and stream were calculated using TOXSWA model with no drift entry. The resulting maximum PEC/RAC ratio for a 1-year period after application was plotted against the SWI on the day of application.

The results show that for herbicide C for scenario D2 Brimstone a potential trigger could be set at 60% SWI. Herbicide B for scenario D6 Thiva has a potential trigger of 85% SWI. For D5 La Jaillière, for herbicide A, B and C, no clear threshold trigger was observed with 10% of the data points not following the pattern observed in the monitoring study; excluding these values, a potential SWI trigger of 85% could be observed.

It can be concluded that the threshold triggers and the magnitude of their effect on drainage losses varied between scenarios and substances depending on their properties. Thus, the decision about the applicability of this approach as a potential drainage mitigation must be done on a case-by-case basis.

### **3.15.P-Mo247 Reduction of Complexity: Variance-based Sensitivity Analysis for FOCUS STEPS**

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To standardize the role of models in the European Union (EU) plant protection product authorization process, Forum for the Co-ordination of pesticide fate models and their use (FOCUS) has established guidelines, including the FOCUS STEPS models for surface water risk assessment. Conservative scenarios representing EU member states are used, with a 4-tier approach for limit value exceedance. Higher-tier calculations, while requiring more information, provide more accurate results. When calculating the respective tiers, however, the accuracy of the model parameters does not have to be specified. This can mean that a parameter to which the model reacts very sensitively - i.e. the result depends relatively heavily on variations of it - can lead to a large inaccuracy.

The project employs sensitivity analysis using the Sobol' method. Model parameters are sampled using low-discrepancy sequences, and Sobol' indices are calculated to determine the sensitivity of the FOCUS STEPS models to parameter variances. The analysis focuses on the first order Sobol' indices representing the main effects, providing insights into parameter influence.

FOCUS STEPS 1 as the simplest example, is presented with a limited set of parameters. The analysis of scenario parameters, such as drift and runoff proportions, field-to-water ratio, and water depth, reveals sensitivity patterns. For low values of the sorption constant  $K_{oc}$ , the model is sensitive to runoff parameters, while higher  $K_{oc}$  values make it more sensitive to drift fraction. With low  $K_{oc}$  leading to high concentrations, the variance of runoff parameters should, hence, be decreased to a minimum. Higher Tiers are handled to some extent afterwards.

Sensitivity analyses help identify influential processes and parameters. The variance-based analysis guides users on reducing uncertainties, especially when concentrations are near regulatory thresholds. Considerations for modifying model user interfaces to suggest variance sensitivity based on input configurations are proposed for enhanced user guidance.

### **3.15.P-Mo248 The Effect of Time-Varying Soil Properties Caused by Ploughing and Consolidation on Pesticide Fate in Soil and Groundwater**

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Soil properties such as the dry bulk density and soil hydraulic parameters can significantly affect the environmental fate of pesticides. These properties are often assumed to remain constant in time in numerical models. However, these properties change over time during ploughing and consolidation in reality. In this study, we modeled the time-varying soil properties induced by ploughing and consolidation and assessed its effect on pesticide accumulation in the topsoil and leaching to the groundwater. For this purpose, time-dependent soil properties have been implemented in the pesticide fate model PEARL. Ploughing instantaneously decreases the bulk density, after which it gradually increases again to its original value through consolidation caused by rainfall. The time-dependent soil properties are modelled based on empirical relationships between the dry bulk density and the Mualem-Van Genuchten parameters found in the literature.

Ploughing leads to a short-term deviation of the soil water content and concentration compared to the reference case (i.e., the case with constant soil properties). We included mixing of pesticide over the ploughing layer due to ploughing in both cases. However, under Central European climate conditions, the effect of ploughing vanishes within several months in the entire soil profile. For assessing the impact on the leaching of pesticide to groundwater, we evaluated the pesticide concentration in pore water at 1 meter depth. The effect of time-varying soil properties due to ploughing and consolidation on the leaching concentration was found to be small for both a tracer and an adsorbing solute. Even for an extreme case with three ploughing events per year, the effect on the 90<sup>th</sup>-percentile of daily leaching concentration was smaller than 0.3%. For assessing the impact on the exposure of soil organisms to pesticides, we considered the pesticide concentration in pore water averaged over the upper 20 centimeters of the soil. For the tracer, ploughing resulted in a 1.2% decrease of the 90<sup>th</sup>-percentile of daily topsoil concentration data for the extreme case of three ploughing events per year. Interpretation of the results for adsorbing solutes in the topsoil is hampered by the fact that soil mass is not conserved in the current approach. More advanced models must be developed that allow for conservation of soil mass for assessing the impact of time-dependent soil properties on concentrations in the topsoil.

### **3.16 Measuring Chemicals in the Environment – Maximising the Utility of Monitoring Data for Environmental Assessment**

#### **3.16.T-01 An Approach for Evaluating the Reliability of Measured Environmental Concentrations in Environmental Risk Assessment**

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The Criteria for the Reporting and Evaluation of Exposure Data (CREED), developed through a SETAC Technical Workshop, are designed to account for all aspects of data reliability, as well as the relevance of the data for the purpose of the assessment; the end result is an overall score for the dataset to be used for that purpose. Through a workshop and meetings held by a group of experts detailed criteria for data reliability were developed, alpha- and beta testing was then conducted to refine the set of criteria. The focus of this presentation is the reliability evaluation contained in CREED.

Prior to completing the CREED reliability (or relevance) assessment, a dataset must meet a minimum set of criteria (called the Gateway Criteria). If the dataset to be evaluated meets the gateway criteria it can be further assessed. If the dataset does not meet the gateway criteria the assessment should be stopped, or the missing information should be obtained (if possible).

The overall reliability assessment consists of up to 19 criteria covering six different aspects: Media, Spatial, Temporal, Analytical, Data Handling and Statistics and Supporting Parameters. The number of criteria requiring completion is dependent on the dataset being assessed. All criteria are answered using the rating system of Fully met, Partly met, Not met/inappropriate, Not reported or Not applicable. It is important that Not met/inappropriate should only be used where the information reported is determined by the assessor to be Not appropriate; if the data are missing then Not reported should be utilised.

Once the criteria have been rated, the dataset is scored into a final reliability category (Reliable Without Restrictions, Reliable With Restrictions, Not Reliable or Not Assignable) at each of two scoring levels, Silver and Gold. The Silver level is less rigorous, as the reliability score is based on only 8 criteria (those designated as required for most assessment purposes). At the Gold level, all criteria (required plus recommended) are included in the scoring, representing an ideal dataset.

The criteria have been designed to allow for consistent and accurate assessment of environmental concentration datasets in risk assessments and when used with the CREED relevance criteria to provide a standardised reporting format for data. CREED may also be used by data generators to identify the information assessors require to ensure that the maximum amount of data generated can be used.

### **3.16.T-02 Assessing the Reliability and Relevance of a Dataset for Evaluating the Occurrence of Hexa(methoxymethyl)melamine in Surface Waters**

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The CREED (Criteria for Reporting Environmental Exposure Datasets) assessment approach provides a framework for evaluating in a systematic and transparent manner both the reliability and relevance of environmental exposure datasets. This assessment approach is applied here to evaluate the reliability and relevance of a combined dataset on the occurrence of hexa(methoxymethyl)melamine (HMMM) in surface waters. More specifically, the dataset was assessed for the purpose of understanding to what extent the substance HMMM occurs in Dutch surface waters and whether temporal trends and regional patterns exist. For the given purpose, the dataset was scored as 'usable without restrictions' at the Silver level i.e., at the level required for data usability. Information on analytical tools was initially not available, making the dataset unusable. Enquiring the monitoring organisation allowed accessing the missing information to reach the silver level for the reliability assessment. With respect to relevance, all the criteria were found fully met. Thus, all data suppliers in the dataset could reach the silver level.

This presentation highlights how applying the CREED approach promotes a more thorough and transparent assessment of exposure data: by establishing clear criteria of usability for a given purpose it encourages data users to ask for and assess additional information missing in the dataset, while encouraging data suppliers to provide the relevant information needed for using the data.

### **3.16.T-03 Trend Analysis of Environmental Concentrations of Bisphenol A in European waters**

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Bisphenol A (BPA) is a high production volume chemical that has been in commercial production for over fifty years. Increasing regulatory scrutiny over the potential endocrine disrupting properties of BPA and other bisphenols has led to proposals for regulatory measures. There are numerous reports of the presence of BPA in environmental media, with the aquatic environment being the compartment of most focus.

We present a robust analysis of measured environmental concentrations of BPA from 2010 to 2022. Monitoring data for BPA in European fresh and saline surface waters and sediments published in peer reviewed scientific literature and publicly

accessible monitoring databases such as the NORMAN network's EMPODAT database were collated. Data were systematically evaluated for relevance and reliability using the Criteria for Reporting and Evaluating Exposure Datasets (CREED) approach, developed by a SETAC Technical Workshop with the aim of fostering consistent and transparent evaluation of chemical exposure data. All relevant and reliable data were compiled into a database. Data are available for 21 countries. Limits of detection (LOD) varied between studies and a significant proportion of the data were reported as less than LOD.

The dataset underwent statistical processing to address censored data and the variability in reported detection limits. Data presented as summary statistics were imputed for the known sample size and observations were weighted to accommodate variations in sampling locations.

Summary statistics (mean, standard deviation) were determined for each environmental compartment and compared to analyses from earlier time periods. Concentrations of BPA varied spatially and temporally between countries. Reported concentrations of BPA ranged over several orders of magnitude in all relevant compartments.

Examination of the data suggested an overall decrease in median concentrations over time in freshwater and freshwater sediment. A noticeable decrease in median BPA concentration in European freshwaters was evident after 2015. Trends in BPA concentrations in individual countries with a substantial dataset were also observed.

### **3.16.T-04 Concentrations of copper in the environment across the EU: an analysis of national copper monitoring data and the influence of land use**

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Copper provides a vital role across a broad range of industries including agriculture, as a fertilizer, growth promotor and fungicide. In recognition of the shortcomings of standard regulatory frameworks to handle transition metals such as copper, the European Food Safety Authority PPR panel published 'a framework for the environmental exposure and risk assessment for transition metals when used as active substances in plant protection products'. This framework expands the role and importance of public environmental monitoring data in support of the environmental risk assessment.

To satisfy these requirements and to support the renewal of copper as a fungicide according to regulation (EC) 1107/2009, all available EU relevant national and transnational copper monitoring data in surface waters, groundwaters, sediments and soils were sourced from regional and national environment agencies/institutes. They were then harmonised and collated into a database suitable for regulatory assessments, which included supplied or assigned landcover attributes using the CORINE landcover dataset. An analysis of these data was undertaken to determine natural and land use specific background copper concentrations for different environmental compartments for use in environmental risk assessment. This included landcovers of particular relevance to fungicidal copper use (e.g. vineyards, orchards and arable cropland). These background values determined were compared to the estimates from other European monitoring programmes such as LUCAS, GEMAS and FOREGS

Key findings include: 1) there is considerable variability in the data quantity, quality and distribution, showing that chemical monitoring data in the EU is not always easily obtained nor sufficiently characterised that it can be integrated into an EU dataset; 2) The data that is reliable for inclusion and use in regulatory assessments can be spatially and temporally variable resulting in small sample numbers for specific countries and/or landcovers; 3) the results from these analyses yield concentrations that are comparable to other estimates such as those derived by the LUCAS topsoil survey.

### **3.16.T-05 Persistence, seasonality and sporadic releases of micropollutants within the urban water cycle - the benefit of four-year monitoring program**

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Regulatory changes reflect the growing understanding of micropollutant impacts in the aquatic environment. By understanding the public health (drinking water) and ecosystem (wastewater) impacts of micropollutants via extensive monitoring, water utilities can actively foster environmental stewardship and positive relationships with community members, business partners, and regulatory agencies. This study spanned 48 months of monitoring along the 95 river km of the Bow River watershed in Calgary, Alberta, Canada. We aim to (1) establish baseline data for water utility operations, (2) enhance our understanding of micropollutant behavior in the urban water cycle, (3) assess the contributions of three wastewater treatment plants (WWTPs) to downstream receiving waters, and (4) prioritize a subset of substances for continuous monitoring. Our results indicate the extensive persistence of metformin (antidiabetic), seasonality of N,N-diethyl-*m*-toluamide (DEET, insect repellent), O-desmethylvenlafaxine (antidepressant metabolite), and sulfamethoxazole (antibiotic) in source waters, and sporadic detections of a well-known perfluoroalkyl substance (PFOA). The seasonality of pharmaceuticals at the sentinel downstream monitoring site appeared to coincide with river dilution while that of DEET was likely attributable to peak usage during the warmer

months. Steroidal estrogens were rarely detected in wastewater effluents although total estrogenicity was evident, suggesting the presence of less potent but more abundant non-steroidal estrogens (e.g., flame retardants, bisphenols, and phthalates). Finally, hierarchical clustering revealed a close association between *E. coli* and caffeine, supporting the use of caffeine as an indicator of short-term, untreated anthropogenic inputs. Overall, this study provides insights on the sources and behaviour of organic micropollutants and identifies indicators useful for prioritizing future monitoring campaigns in Calgary and elsewhere.

### 3.16.P Measuring Chemicals in the Environment – Maximising the Utility of Monitoring Data for Environmental Assessment

#### 3.16.P-Th321 Regression on Order Statistics – An Improved and Straightforward Approach to Dealing with Non-Detects?

*Iain Wilson and Mike Gardner, wca environment Ltd., United Kingdom*

Treatment of data reported as below the limit of detection (LoD) represents a challenge when performing a chemical risk assessment. Numerous methods of dealing with this have been applied, ranging from simple (non-statistical) approaches, for example ignoring censored values or using the half LoD method (also known as substitution), to complex statistical methods such as Maximum Likelihood Estimation and Kaplan-Meier analysis. What appears to be required is a proven statistical method that can be readily applied. The method of Regression on Order Statistics (ROS) is such an approach that addresses the potential defects of either questionable simplicity or off-putting complexity.

To apply the ROS method, the distribution of the dataset to be analysed is required to be determined, typically for environmental datasets they are found to be lognormally distributed. Once the distribution has been identified, the natural logarithm of data is calculated, the transposed data is sorted in ascending order, with blanks for the censored values, and rank orders are created. Q-Q plots are produced and the y-intercept of the straight line and the slope provide the population mean value and standard deviation, respectively. These values can be re-transformed to real world values based on defined equations, and used as the basis of the (estimated) uncensored data distribution in a cumulative frequency distribution (CFD). In the CFD the data distribution of the non-detect observations can be plotted using the inverse lognormal data distribution versus the median rank values corresponding the non-detect values. This “invented” section of the CDF is not strictly part of the ROS output and is shown merely as an aid to the visualisation of the distribution of lower percentiles. This method has been shown that it reduces both systematic and random errors of the mean compared to the substitution method.

As complex as this sounds, this poster will show that it can be applied relatively easily using basic tools. Additionally, using real-world data the affect the use of ROS can have on the estimation of the mean when a dataset includes censored data compared to the substitution method on datasets of differing degrees of censorship and how this can potentially impact the risk conclusion to European surface waters will be demonstrated. This approach is designed to show that ROS can be applied by non-statistical experts and provide greater confidence in results from censored datasets.

#### 3.16.P-Th322 AQUA-GAPS/MONET-derived concentrations and trends of PAHs and polycyclic musks across global waters

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The AQUA-GAPS/MONET network was established to provide global trends of organic contaminants in the waters of the world while relying on the same passive sampling material analyzed by a central laboratory to ensure quality control. Prior work reported on concentrations, global distributions and trends of organochlorine compounds (PCBs and OCPs). In here, we discuss the presence of polycyclic aromatic hydrocarbons (PAHs) and polycyclic musks (PCMs) derived from the same set of passive samplers. The detection frequency of the targeted PAHs ranged from 35% (cyclopenta[cd]pyrene) to 98% (triphenylene). Seven PAHs were present in > 90% of all sampling events: triphenylene, chrysene, retene, benzo[*g,h,i*]fluoranthene benzo[*b*]fluoranthene, fluoranthene, and benzo[*e*]pyrene. There was a general trend of decreasing aqueous concentrations with an increasing molecular mass. The sum of 28 PAHs (S<sub>28</sub> PAHs) concentrations ranged from 61 – 45,000 pg/L, with mean (median) concentrations of 7,200 pg/L (3,200 pg/L). Greatest concentrations of phenanthrene, fluoranthene and pyrene were typically from the same sites, mostly in Europe and North-America. While the exact ranking varies by compound, similar trends applied to several more PAHs, including anthracene, pyrene, fluoranthene and benz[*a*]anthracene. These PAHs are typically associated with the incomplete combustion of (fossil) fuels. Different geographical abundances were present for retene, which ranged from ND – 490 pg/L, with greatest concentrations present in water bodies from Chile, Brazil, Norway and remote lakes in Slovakia. The presence of retene is typically interpreted as an indication of wildfires/the burning of coniferous biomass. Of the 6 PCMs, only galaxolide (HHCB, detected in 72% f samples) and tonalide (AHTN, 61%) were regularly detected. Maximum concentrations reached up to 20,000 pg/L for galaxolide and 1,900 pg/L for tonalide. Concentrations of 11 of 28 PAHs, galaxolide and tonalide were positively correlated ( $P < 0.05$ ) with population density within a radius of 5 km of the sampling site. There were no significant correlations between any of these PAHs or PCMs and temperature, latitude or longitude – implying the lack of global fractionation or physicochemical properties on the distribution of PAHs or PCMs. This was not surprising, given the known reactivity of these compounds, and their multiple active sources with different emission rates and patterns around the globe.

### **3.16.P-Th323 Effectiveness and Interpretation of Surface Water Chemical Quality Indicators: A Case Study in England.**

*Neve Hughes, University of Birmingham, United Kingdom*

The evaluation of the Water Framework Directive (WFD), in relation to the surface water environment, in 2019 revealed that all England's surface waters have failed to meet a good standard for chemical quality. The 25-year environment plan (25YEP) seeks to improve the environmental health of the UK over the next 25 years and includes new indicators to monitor progress. This study analysed different indicators used to monitor chemical quality of surface waters, including the H4 pesticides in surface water indicator of the 25YEP.

The case study focuses on assessing the chemical quality of England's surface water bodies using data gathered from the Environment Agency (EA) water quality monitoring (WIMS) database, specifically their semi-quantitative gas chromatography-mass spectrometry (GCMS)/ liquid chromatography-mass spectrometry (LCMS) dataset. The chemical water quality indicators were developed using this data for the period 2007 - 2021. The indicators were assessed on how factors including spatial, temporal and substance specific factors impact the indicator in order to draw conclusions as to their effectiveness and potential issues to be aware of during interpretation.

The indicators are impacted by number of samples; number of substances analysed; changes in substance availability and use; seasonal and annual variations and changes in chemical pressure. It is important to have reliable indicators of chemical water quality to track progress of policies given the increasing pressure on water resources due to overuse and deterioration of quality and this study provides insights into indicators that are currently used and makes recommendations for their improvement and interpretation.

### **3.16.P-Th324 LUCI - A GIS-tool to select monitoring sites in river networks based on catchment properties allowing for hypothesis testing and statistical independence**

*Tom Galle, Christian Braun and Philippe Pinheiro, Erin, Luxembourg Institute of Science and Technology (LIST), Luxembourg*

Regulatory monitoring of surface waters is often unfit to derive diffuse pollution inputs from the landscape because the sites of measurement are located at confluences of rivers with limited land use property variability. Statistical analysis of land use vs exposure/load of pollutants is therefore often very weak or nonexistent. In order to investigate land use impacts on surface water pollution we developed a web-based GIS tool that allows for the filtering of monitoring points in river networks that represent specific land use properties and consequently pressures on surface waters. The tool allows to build gradients of pressures by choosing different levels of land use or activities in a catchment while being able to exclude others. This approach strengthens statistical power of the analysis by building gradients of expected impact (moving to extreme land use) and sampling independent sites (avoiding longitudinal profiles which are auto-correlated). Hence plausibility of results can also be investigated through the array of independent monitoring sites. The contribution will illustrate the use of the powerful tool with examples of sealed surfaces in metal and PAH pollution of low-flow suspended sediments, the pharmaceutical/biocide footprint of waste water treatment plants in catchments of different sanitary pressure and the link between maize cultures and herbicide impacts on macrophytes in agricultural headwaters.

### **3.16.P-Th325 The challenges of creating a pan-European database and open-access dashboard of readily available public chemical monitoring data**

*Gregory Hughes, GeoSpatial Analytics Ltd, United Kingdom*

Access to public environmental monitoring data has improved in the last two decades, largely owing to open data initiatives linked to the public access to environmental information regulation (2003/4/EC). These data are collected by public bodies, like environment agencies, or organisations working in the public interest, like water/utility companies, to meet regulatory obligations and research objectives according to regional/national guidelines for sample collection, storage and analysis. In short, the data is collected by different organisations, for different purposes in different ways which makes creating a harmonised database at a pan-EU scale challenging. However, this is increasingly required for regulatory purposes, for example the assessment of chemical quality data for a range of environmental compartments under the Water Framework Directive and Plant Protection Products regulation (1107/2009/EC).

In some cases, the chemical quality data is readily available online but takes many different forms ranging from pdf documents/Excel spreadsheets and zipped data archives through to elaborate web-portals that not only provide access to the data but allow the user to interrogate the data and display data summaries. In many cases, the data need to be requested and in some cases a data extraction fee paid. Much of the data is available under an open-data type of license but in some cases, where third party data is involved, use is restricted through a specific user agreement. The geolocation of monitoring sites is provided in different projections and at different spatial accuracies depending on location security policies, especially for drinking water supply abstractions. The format of the data is highly variable requiring significant effort to harmonise the data into a coherent curated database suitable for regulatory use.

This poster outlines the collection and transformation of readily available public chemical quality data into information with the creation of a curated, harmonised, SQLite based public environmental monitoring database using a range of techniques,

including the use of python based web-scrappers. It also documents the leverage of this information into knowledge through an open access R-Shiny dashboard that allows pan-EU summaries of chemical water quality data to be interrogated. The utility of easy access to pan-EU environmental information and its role in promoting sustainable practices whilst protecting the environment is outlined.

### **3.16.P-Th326 Comparison of the relevance of a dataset for different assessment purposes**

*Adam Peters<sup>1</sup> and Jeanne Vuaille<sup>2</sup>, (1)wca environment Ltd., United Kingdom, (2)European Environment Agency (EEA), Denmark*

The CREED (Criteria for Reporting Environmental Exposure Datasets) assessment approach provides a framework for evaluating both the reliability and relevance of environmental exposure datasets. Whilst the reliability of any individual dataset is independent of its purpose, the relevance of a dataset is always purpose-dependent and different criteria are therefore required for different assessment purposes. This presentation compares the relevance of a single dataset for three different, but related purposes and aims to highlight the important differences in the data requirements for the different purposes. The different assessment purposes are (1) the prioritisation of a substance for Environmental Quality Standard (EQS) development, (2) deriving a bioavailability based environmental quality standard, and (3) evaluating whether a substance still poses a sufficiently widespread problem for a statutory environmental quality standard to still be required. The prioritisation is focused on exposure data, but given that this occurs before the derivation of an EQS the requirements for the limits of quantification and spatial representation of data may be less stringent than for other purposes. The derivation of a bioavailability based EQS is focused on the sensitivity of the surface waters to identify sensitive water types, and not on the exposures themselves. An assessment to evaluate the continuing need for a statutory standard has specific requirements in relation to the timescales, the representativeness of the sampling, and the spatial coverage, as well as the limits of quantification. These differing requirements result in differing levels of relevance of a given dataset for the different purposes. The assessment is performed using a single dataset from the UK to illustrate these issues.

### **3.16.P-Th327 Assessing the Reliability and Relevance of a Dataset for Evaluating the Effects of Metal Mixtures on Benthic Macroinvertebrates in the Field**

*Adam Peters<sup>1</sup>, Iain Wilson<sup>1</sup>, Graham Merrington<sup>1</sup>, Jeanne Vuaille<sup>2</sup> and Yamini Gopalapillai<sup>3</sup>, (1)wca environment Ltd., United Kingdom, (2)European Environment Agency (EEA), Denmark, (3)International Copper Association, Ltd.*

The CREED (Criteria for Reporting Environmental Exposure Datasets) assessment approach provides a framework for evaluating both the reliability and relevance of environmental exposure datasets. This assessment approach is applied here to evaluate the reliability and relevance of a combined dataset of chemical and ecological data that is used for assessing the effects of metals and metal mixtures on benthic macroinvertebrates and their communities in the field. Although the CREED assessment approach was developed specifically for data on chemical exposures it is extended here to also include information on the freshwater habitat at sampling sites and the abundance of benthic macroinvertebrates, although in a more limited manner. This application of the CREED assessment approach demonstrates that clear, transparent, and auditable assessments of reliability and relevance can be applied to other kinds of environmental data beyond purely chemical measurements. This presentation highlights some of the limitations of the CREED approach, particularly as applied to these kinds of data that it was not originally generated for, and draws conclusions about the suitability of the dataset evaluated for evaluating the effects of metal mixtures on freshwater invertebrate communities.

### **3.16.P-Th328 Temporal Trends of Atmospheric PBDEs in Spain: An Analysis over a Decade (2008-2019)**

*Juan Munoz-Arnanz, Pere Colomer Vidal, Maria Ros, Alba Vicente, Cielo Salcedo, Estela Nogales and Begoña Jiménez, Institute of Organic Chemistry, Spanish National Research Council (IQOG-CSIC), Spain*

Polybrominated diphenyl ethers (PBDEs) are among the dominant flame retardants used for decades. This class of Persistent organic pollutants (POPs) are regulated under the Stockholm Convention (SC). This study, within the framework of the Spanish Monitoring Program on POPs since 2008, focuses on assessing PBDE concentrations in the air in urban and background areas in Spain to evaluate the effectiveness of the measures taken under the SC. PBDEs were analyzed by GC-HRMS in more than 500 air samples, obtained with passive sampling devices during a decade.

Despite fluctuations in measured concentrations, a consistent pattern of relative abundance in the air emerged: PBDE-209 predominantly contributed to  $\Sigma$ PBDEs, followed by PBDE-47 and PBDE-99 in both urban and background sites. Urban areas exhibited notably higher median concentrations than background locations, with significant differences observed between various urban and background locations.

Within the study period, a positive correlation between time and global PBDE concentrations was detected, indicating an overall increase in PBDE levels. Interestingly, the correlation of only PBDE-209 with time was just marginally ( $p=0.055$ ) significant, while the sum of the remaining PBDEs did not exhibit a significant positive correlation with time. Moreover, minor contributors (BDE-17, 28, 66, 85, 100, 153, 154, 183, 191, 196 and 197) showed a significant negative correlation, suggesting a decrease over time.

Location-specific trend analysis revealed time-concentration relationships with  $\Sigma$ PBDEs in various cities. Significant positive correlations were observed in several urban and background areas, while a negative correlation was found at one background



area. Similar correlations were found for PBDE-209 in almost all locations, underscoring the high influence of this congener on the general trends.

Our chemical monitoring dataset underscores how PBDE temporal trends are highly location-dependent, emphasizing the importance of long-term monitoring and location-specific influence in assessing atmospheric PBDE pollution. The divergence in the PBDE congener abundance and the significant variations among locations highlight the necessity to better understand pollution patterns in the environment to support chemical regulation and their implications for human and environmental health.

### **3.16.P-Th329 Contextualisation of RAC exceedances reported for small surface water bodies in Germany**

**Oliver Körner<sup>1</sup>, Carola Schriever<sup>2</sup>, Herbert Ressler<sup>3</sup>, Andreas Solga<sup>4</sup>, Stefan Kimmel<sup>5</sup>, Judith Neuwöhner<sup>6</sup>, Stephan Partsch<sup>7</sup>, Sophia Müllner<sup>8</sup> and Mark Winter<sup>8</sup>, (1)ADAMA Deutschland GmbH, Germany, (2)BASF SE Agricultural Solutions, Germany, (3)Syngenta Agro GmbH, Germany, (4)Bayer AG - Crop Science Division, Germany, (5)Corteva Agriscience, Germany, (6)Nufarm Europe GmbH, Germany, (7)FMC Agricultural Solutions, Germany, (8)Industrieverband Agrar e.V., Germany**  
A surface water monitoring campaign (2018-2019) was commissioned by the German Federal Environment Agency (UBA) to assess the chemical and biological condition of small agricultural watercourses and verify the effectiveness of risk assessment and management in the pesticide approval process. The final campaign report concluded that regulatory acceptable concentrations (RAC) of pesticide active substances were exceeded in more than 73% of the investigated watercourse sections, indicating a general underestimation of the actual risk in pesticide approval.

The German Plant Care Industries Association (IVA) evaluated the publicly available raw data from the campaign to understand the causes for the reported RAC exceedances. The IVA found that for approximately 75% of active substances with RAC exceedances listed in the final report, the considered RACs (UBA-RACs) differed from the legally binding RACs valid at the time of approval (BVL-RACs; BVL = Federal Office of Consumer Protection and Food Safety). Considering the BVL-RACs, the total number of exceedances is approximately 50% lower compared to the final report of the campaign. UBA-RACs were lower due to various reasons: additional safety factor of 3 was applied, supportive (higher-tier) studies (e.g., metamitron or 2,4-D) or data from EU documents (e.g., acetamiprid) were not considered.

Reviewing the concentrations from the campaign report, the IVA observed that for the majority of analysed active substances, more than 95% of the water samples contained concentrations below the BVL-RACs. Only three active substances exceeded BVL-RACs in more than 5% of the samples with two of them being also used in the non-agricultural sector and being detected alongside waste water markers. In conclusion, the campaign results suggest that surface water exposure under real world conditions is below levels of concern for the vast majority of analysed active substances and sites, indicating a functioning pesticide approval process.

### **3.16.P-Th330 Monitoring chemical emissions from offshore wind farms: assessing impacts, gaps and opportunities**

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Offshore wind energy offers many advantages to society as well as the marine ecosystem. Next to the primary aim of producing renewable energy, offshore wind farms (OWFs) also offer opportunities to combine energy production with other activities such as nature conservation or aquaculture. The vast increase in offshore energy production urged different European countries to monitor the potential effects of OWFs on the marine ecosystem, including the effects of underwater noise and the introduction of novel habitats, but also the exclusion of fisheries. However, the potential risk of chemical emissions by OWFs remains largely unknown.

Chemical emissions from OWFs may be divided into three groups: metals, organics and particles. OWF turbines and substations contain corrosion protection systems which can leach metals such as aluminium or zinc into the ocean. Organic chemical emissions are expected from different sources such as leaching from paints, leakages of transformer and lubricating oils, and the unintended release of fire extinguishing agents. Particulate emissions result from the release of paint flakes and the abrasion of small composite and plastic parts from the rotor blades. To the best of our knowledge, no significant risks from OWF chemical emissions into the marine ecosystem have currently been identified.

Within the Interreg North Sea Region project Anemoui, the aim is to assess impacts, knowledge gaps and opportunities related to chemical emissions of OWFs. In this poster presentation, an overview of potential emission sources will be provided and the potential challenges to monitoring and forecasting will be addressed. These challenges include measuring low concentrations of a diverse set of pollutants in marine compartments, estimating the relative share of emissions originating from OWFs compared to other marine sources and predicting the behaviour of particles in a dynamic marine environment.

First data of contaminants measured in sediment samples taken in/near OWFs will be presented and future steps toward emission reduction outlined.

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### **3.16.P-Th331 Using biota monitoring data to identify priority substances and improve chemical risk assessment and management under REACH**

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Most chemicals are registered under REACH. There the traditional environmental risk assessment is based on predicted environmental concentrations (PECs), usually in water, to be compared with predicted no-effect-concentrations (PNECs), usually derived from algae, daphnia and fish. Only for high tonnage industrial chemicals, also sediment and terrestrial toxicity data are available. Therefore, exposure and effects in higher trophic level species and on terrestrial organisms are often not assessed, as seldom data are available - at least under the European REACH regulation. Biomonitoring data offer a direct insight into which substances organisms are exposed to and, additionally, can be used to perform the bioaccumulation assessment within REACH and CLP. Therefore, environmental biomonitoring data can give a more realistic estimate of the actual exposure level of organisms than traditional exposure assessments, especially when more species are analysed at the same location and time. However, interpreting internal chemical concentrations in the context of toxicity assessments within regulatory risk assessment - at organism or even at ecosystem level - remains challenging. Particularly, since systematic approaches on how to make use of biota monitoring data on this vital aspect are scarce. To address this knowledge gap, the German Environment Agency (UBA) is actively engaged in several projects aiming at constructing a robust database and bridging the gap from internal concentrations to adverse effects on organisms and ecosystems. Most prominent these are the HORIZON TerraChem and the UBA funded Beyond Life Apex project. Results will be used (1) to identify and prioritize chemicals that are driving biomagnification, toxicity and ecological risks, and (2) to enhance current risk assessment methods and management practices. This poster will outline UBA's efforts in leveraging biomonitoring data, display the first results from Europe wide biota monitoring and open the door for discussions on how to shape a more effective regulation and biodiversity protection.

### **3.16.P-Th332 Copper does not appear to drive adverse effects on German aquatic biodiversity: A spatial analysis of copper concentrations in German surface waters and sediments and biomonitoring data**

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Copper and copper compounds have been a vital fungicidal tool in agriculture for over a century and continues to be of key importance, especially for organic farming where a more limited range of fungicides are available to support crops. In addition to fungicidal use, copper is a key component of fertilizers, biocides and has many other industrial applications leading to a continuous input into the environment. Additionally, copper, a transition metal, is nutritionally essential, but toxic in high doses and non-degradable. Recognizing this, the European Food Safety Authority (EFSA) published a statement on the environmental risk assessment for transition metals that expands the role and importance of monitoring data.

In order to examine the impact of copper on aquatic wildlife, we performed a spatial analysis combining publicly available copper monitoring data (extracted from a database of all national monitoring and catchment based monitoring programs) and biomonitoring data in German surface waters and sediments. The analysis aimed to assess if there were any adverse effects on the biodiversity in German freshwater ecosystems due to the presence of copper. Specifically, Generalized Linear Mixed Models were used to identify any correlations between the number of species of fish, macrophytes and phytobenthos, phytoplankton or macrozoobenthos and localised copper concentrations.

Our key findings are: 1) median measured copper concentrations in surface waters were 2.4 µg/l and 2.1 µg/l, for total and dissolved copper respectively; 2) median measured copper concentration in German sediments was 49.0 mg/kg; 3) no correlations were found between copper concentration and macrophytes, phytobenthos, phytoplankton or macrozoobenthos species; 4) a minor increase in the number of fish species was associated with increasing copper concentrations although this is most probably an indirect effect due to other environmental influences; and 5) monitoring and biomonitoring data are not always easily accessible nor are they consistent between programmes causing complications with spatial and temporal representativity. Consideration must be put into a more unified approach to make use of what is otherwise a large and highly

valuable dataset. Overall, this analysis shows that copper in German surface waters and sediments does not appear to be a key driving force number of aquatic taxa.

### **3.16.P-Th333 Determination of Biogenic Sterols in Surface Sediments of Admiralty Bay, Antarctic, and their Relationship with Recent Environmental Changes**

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The Antarctic Continent is considered one of the most untouched areas on the planet due to its geographical location and distance from populated regions. In recent decades, the Antarctic Peninsula has experienced a sharp rise in temperature, being higher than the global average. The main consequence of this change is linked to the loss of area and volume of the glaciers, generating a greater discharge of continental material to the glaciomarine environment, thus bringing changes in the dynamics of carbon in the area. Sterols are important biogeochemical tools applied to understand the sources, transport, and fate of organic compounds in the marine environment. In addition, they act as fingerprints of primary production at different time scales. Thus, the present work seeks to investigate the spatio-temporal distribution of geochemical markers (sterols) as indicators of origin, variations in input, preservation, and degradation of sedimentary organic matter in samples of surface sediments. Sampling was carried out along the three major inlets of the bay, namely: Martel, where the Brazilian station Comandante Ferraz (EACF) is located, Mackellar, where the Peruvian station Machu Picchu is located, and Ezcurra, where the Polish station Henryk Arctowski is located, during 2002 - 2018. The sterols were analyzed by gas chromatography with a flame ionization detector after Soxhlet extraction, purification in the liquid adsorption column, and derivatization reaction. The results obtained suggest that in this environment there is a multiplicity of marine sources and the variability of the concentration over time may indicate a predominance or decline of producing species in the face of environmental changes. Cholesterol was the dominant sterol ( $2.85 \pm 1.63 \mu\text{g.g}^{-1}$ ), and the contributions of penguins and seals feces as well as inputs associated with phyto and zooplankton explained this trend. Sitosterol showed an average concentration of  $1.42 \pm 0.53 \mu\text{g.g}^{-1}$ . Brassicasterol and stigmaterol were detected in practically all samples along the time series, with averages equal to  $0.84 \pm 0.23$  and  $0.47 \pm 0.19 \mu\text{g.g}^{-1}$  respectively, evidencing the contribution of diatoms, cyanobacteria, and dinoflagellates to the buried organic matter in marine sediments. In addition, the concentration differences between inlets may also reflect the variation in the abundance of organisms in different locations, indicating that their distribution in the environment is not always homogeneous.

### **3.16.P-Th334 Estimation of Gas-particle partitioning Coefficients (Kp) of POPs in different Antarctic region.**

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Gas/particle (G/P) partition, describing the distribution between the gas and particle phase in atmosphere, is an important influencing factor for the deposition, chemical reaction, and the long-range transport of semi-volatile organic compounds (SVOCs). Another hand, some of the persistent organic pollutants (POPs) reach Antarctic atmosphere through long-range atmospheric transport. In this study, the concentration of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in air samples (gaseous and particulate phase) was determined at four sampling points in Antarctica during the Austral summer from 2019 to 2022. The samples were collected using a high-volume air sampler (MCV: CAV-A/HF, Collbató, Spain) and quantified using Gas Chromatography-Mass Spectrometry (GC-MS). The concentration ranges of OCPs in the gaseous and particulate phases were 8.26-64.15  $\text{pg/m}^3$  and 0.30-1.32  $\text{pg/m}^3$ , respectively. For PCBs, the ranges were 0.12-9.62  $\text{pg/m}^3$  and 0.08-0.26  $\text{pg/m}^3$ , while for PAHs, they were 0.32-5.93  $\text{ng/m}^3$  and 0.04-0.60  $\text{ng/m}^3$ , respectively. The gas/particle partition coefficients (KP) for each sampled compound were calculated as the concentration in the particle phase divided by the product of the concentration in the gas phase and the total suspended particle (TSP) concentration. The results obtained demonstrated that the analysis of atmospheric samples of persistent organic pollutants reveals that these substances tend to predominate in the gaseous phase rather than the particulate phase. The preference for the gaseous phase has significant implications for the dispersion and transportation of these pollutants in the atmosphere, which can influence their effects on human health and the environment and facilitating their long-distance transport. The atmosphere is the dominant transport pathway for these chemical substances at these latitudes. These findings underscore the importance of understanding the distribution of pollutants in different atmospheric phases for a more comprehensive assessment of their impact and to inform effective strategies for pollution management and control.

### **3.16.P-Th335 Differences in the concentrations of organic pollutants in suspended particulate matter in Admiralty Bay, Antarctic Peninsula, during the summer of 2022/23**

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Organic pollutants, such as Polychlorinated Biphenyls (PCBs) and Polycyclic Aromatic Hydrocarbons (PAHs), are introduced into Antarctica through local tourism, fishing, and research activities or transported over long distances. Along the coastal regions of Antarctica, organic compounds in snowmelt can be transferred to seawater during the austral summer melting. Thus,

this study aimed to determine the variations of PCBs and PAHs in the water column of Admiralty Bay, located on King George Island, Antarctic Peninsula, within a short-time scale perspective (from late spring to late summer). Sampling was conducted in five campaigns during the 2022/2023 summer at 16 sites. Approximately 20 liters of surface water were vacuum filtered through GF/F Whatman® (0.7 µm) filters to obtain the suspended particulate matter (SPM). Identification and quantification of 51 PCBs and 16 priority PAHs were performed using an Agilent gas chromatograph model 7890B coupled with an Agilent triple quadrupole TQMS model 7010B operating in multiple reaction monitoring. PCBs contents in SPM varied from 2.64 to 78.15 ng g<sup>-1</sup>. The November and December 2022 campaigns were statistically different from the campaigns of January to March 2023 (Kruskal-Wallis non-parametric test and Mann-Whitney post hoc test;  $p < 0.01$ ). PCBs concentrations were higher in the latter campaigns, coinciding with the increase in snowmelt in late summer. PAHs values varied from 486.3 to 4323.0 ng g<sup>-1</sup>. The February 2023 campaign was the only one that had a significant increase in PAHs concentrations (Kruskal-Wallis non-parametric test and Mann-Whitney post hoc test;  $p < 0.05$ ). After decades of emissions, PCBs have accumulated in soils and snow/ice. Therefore, when the ice melts, the input of PCBs into the water column increases. In contrast, this increase is masked for PAHs since they are currently introduced into the environment through research station operations and ship traffic present in Admiralty Bay. Spatially, PCBs and PAHs showed no significant statistical variations (Kruskal-Wallis non-parametric test;  $p > 0.05$ ) among the three inlets of the bay (Ezcurra, Mackellar, and Martel). The spatial distribution of these pollutants in the SPM of Admiralty Bay is homogeneous, probably due to the strong influence of wind speed on the movement and mixing of the water in this area. This study contributes to the scientific basis for understanding the cycles of PCBs and PAHs in marine environments.

### **3.16.P-Th336 Detection of Particulate and Metal(loid) Emissions from Offshore Wind Farms**

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Current global threats, in particular climate change and geopolitical events, have increased demand for a rapid transition to renewable energy sources. In response, many nations have set ambitious goals concerning the development and installation of energy-generating technologies, including offshore wind farms (OWFs). However, information is still lacking in terms of the long term chemical impacts of these structures and their operation on the marine environment. The goal of this study is to investigate the levels of chemical contaminants within OWF and nearby sites, and to evaluate their traceability to OWF-based sources such as passive and active corrosion protection strategies.

The analyzed sample set originates from a 2023 campaign in the North Sea. Locations within the boundaries of multiple OWFs were sampled in addition to nearby and reference locations with varying levels of anthropogenic impact. Particulate samples were taken from seawater using a filtration cascade down to a filter size of 10 µm. In the laboratory, samples are reduced to a single filter and subjected to two-step digestion and density separation prior to analysis via laser direct infrared (LDIR) imaging. Inorganic seawater and sediment samples to be analyzed with inductively coupled plasma tandem mass spectrometry (ICP-MS/MS) were taken at all particulate sample sites and additional locations.

The abundance and distribution of particulate pollutants including microplastics within OWFs is at the current time virtually unknown and data concerning metal(loid) distributions in OWF areas are still scarce. This study aims to provide initial data on the presence of both OWF-related particles and to implement proposed methods of tracing OWF impacts. The data may be combined with further sampling campaigns, laboratory studies, and toxicological data to obtain an initial perspective on the relevance and magnitude of the chemical emissions and therefore unwanted side effects from renewable offshore wind energy production. Due to planned increases in global offshore capacity, this study is of relevance to regulatory and industry organizations in understanding and mitigating chemical impacts on the marine ecosystem in upcoming decades.

### **3.16.P-Th337 Footprints of Biohazards (VOCs, Volatile Organic Compounds): Monitoring of wastewater**

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The presence of volatile contaminants needs to be monitored at regular intervals to understand the implications on human health and diverse ecosystems. These purgeable incorporate a huge class of chemical compounds, some of which are benzene, toluene, ethylbenzene, and xylenes (BTEX), and other carcinogenic compounds like trichloroethene (TCE), perchloroethylene (PCE), and vinyl chloride. These volatiles enter the groundwater via varied natural as well as anthropogenic (industries, oil spillages, and automobile exhaust) sources which in turn causes contamination. Therefore, an uncontrolled discharge of these VOCs may lead to long-lasting effects on the water bodies which imbalances the biodiversity. To provide sufficient evidence of the presence of these VOCs, this work concentrates on one such case study that particularly focuses on monitoring TCE and PCE in wastewater. Continuous surveillance of TCE and PCE was achieved using a Headspace-trap (HS-trap) technique coupled with the Gas Chromatography-Mass Spectrometry (GC-MS). This sophisticated instrument is proven to have the ability to analyse and precisely report accurate levels of these known carcinogens at sub-below ppb levels. The findings revealed that the levels of these biohazards in varied locations are more than 50% higher than the globally accepted levels for these VOCs, as per WHO regulations for drinking water. Hence, continuous monitoring of these discharged chemicals needs to

be continued over longer periods considering their moderate volatility as well as their consistently higher levels. Further, single source-oriented sampling can help in strengthening their presence as well as provide scientific signatures towards understanding their trend of release into the wastewater systems. Nevertheless, the obtained levels for these volatiles will support the local environmental agencies to curb their release as well as provide control measures to minimize their hazardous impact on ecosystems.

### **3.16.P-Th338 Toxicity of Wastewater with Elevated Bromide concentration**

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Wastewater management is a critical concern for safeguarding our environment and public health. In recent years, the scientific community has been increasingly focused on understanding and mitigating the impact of various contaminants present in wastewater. One such contaminant that has gained attention is Bromide. Bromide is a naturally occurring element that can be found in various water sources, including freshwater and seawater. It is also a common constituent of wastewater, often originating from coal-fired power plants, industrial processes, agricultural runoff, and domestic sources. The presence of bromide in wastewater is of particular concern due to its potential to form disinfection byproducts (DBPs) when it comes into contact with disinfectants like chlorine during the water treatment process. These DBPs can have adverse effects on public health and the environment. Our investigation examines the concentration of bromide in wastewater samples over time, with a focus on identifying spikes or unusual patterns. By doing so, we aim to better understand the sources, effects and associated exposure and health risks.

### **3.16.P-Th339 Presence of anthropogenic contaminants in urban groundwater wells**

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Warranting that drinking water resources are secure from unwanted and toxic chemicals is a central goal of human health protection and human rights, and it is one of the Sustainable Development Goals of the United Nations. One of the water sources considered is groundwater, since its operating costs are lower than those of seawater. However, numerous chemical compounds are used in our daily lives and many of them are not efficiently eliminated in wastewater treatment plants, giving rise to the presence of anthropogenic contaminants in large bodies of water such as rivers, putting the viability of groundwater at risk. In this work, more than 80 anthropogenic contaminants of environmental interest belonging to various groups were selected: drugs, agrochemical compounds, industrial compounds and transformation products. These compounds were analyzed in the Besos River (Barcelona, Spain) and in 16 nearby wells. Approximately, 85% of target analytes were detected in concentrations ranging from 1 to 338 ng·L<sup>-1</sup>, depending on the well and the compound. Furthermore, 60% of the compounds were detected in more than 75% of the samples, showing a constant pollution.

### **3.16.P-Th340 Introduction to Automated Analysis of Multiclass Biohazardous Chemicals (BCs) and Persistent Organic Pollutants (POPs) in Water by APGC-MSMS with RTC PAL SPME Arrow**

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Industrial development and widespread usage of chemicals lead to an anthropogenic environmental pollution. Deliberate or unintentional disposal of these Biohazardous Chemicals (BCs) and Persistent Organic Pollutant (POPs) into biosphere has increased in the last half century. Because of the highly carcinogenic and bio-accumulative nature of these chemicals, they pose a serious threat to the environment and humanity. These compounds are further classified as Volatile Organic Compounds (VOCs), Poly-Aromatic Hydrocarbons (PAHs), Organo-Chlorine Pesticides (OCPs), Poly-Chlorinated Benzenes (PCBs), Phenols, Phthalates, Dioxins, and other chemicals. All these chemicals are analysed using traditional EI-GC-MS/MS, with Liquid-Liquid Extraction (LLE) or Solid Phase Extraction (SPE). However, both the instrumental and sample preparation with these techniques comes with limitations with respect to selectivity for molecular ions, time taking sample preparation, use of larger volume of samples, solvents, and CRMs. The objective of this study is to introduce solvent free automated analysis of BCs and POPs. Solid Phase Micro-Extraction (SPME) is a green i.e., solvent free sample preparation technique, which involves automated extraction and pre-concentration of the analytes by ad/absorption on SPME arrow phase and injection of the analytes by desorption in GC inlet. This technique is evaluated for the Ion Ratio, Coefficient of Determination (r<sup>2</sup>), Precision (%RSD), % Recovery for SPME performance check standard and 20 selected multi-class BCs and POPs using RTC-PAL with SPME Arrow (DVB/PDMS) by APGC-MS/MS. The results of intra-day precision for compounds in mix i.e., 2 Nitrotoluene and Nitrobenzene is ranging from 3.91 to 8.98 and inter-day precision is 10.54 and 12.45 which is within acceptable criteria of 20% RSD. Further, the results for selected 20 multi-class (VOCs, Phenols, Phthalates, PAHs, OCPs and Dioxin) are acceptable. Results for % recovery at 1 ppb spike level (2,3,7,8 TCDD @ 0.05ppb) in water (ASTM Type I) is ranging 85.58 to 111.38 and precision is ranging from 2.30 to 14.28. Therefore, it is evident from the results that with the pragmatic and systematic approach for method development and use of the Internal Standards (IS), automated SPME arrow-based extraction can be considered as tool for qualitative and quantitative analysis of Multiclass BCs and POPs.

### **3.16.P-Th341 Analysis of Difficult Test Chemicals in Support of Aquatic Toxicity Studies (Organization for Economic Cooperation and Development Guidance Document 23)**

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The Organisation for Economic Co-operation and Development (OECD) is an international organisation that works to build better policies for better lives. One of their Guidance Document on Aqueous-Phase Aquatic Toxicity Testing of Difficult Test Chemicals (GD 23) provides additional guidance and approaches for aquatic toxicity studies for difficult test chemicals.

Typical difficult test chemicals are poorly water-soluble or unstable test chemicals in aqueous phase. Multi-component test chemicals or Unknown/Variable composition, complex reaction products and biological materials (UVCBs) are also considered as challenging test chemicals. Such difficulties require a specific approach for aquatic toxicity testing as well as analytical support to test the aquatic media for presence of correct test chemical and correct concentration levels.

To challenge difficult test chemicals in a more structured manner, historical studies have been evaluated to consider logical and successful decisions in study approach. In combination with the OECD guidance document, this evaluation resulted in standardized procedures for both the aquatic toxicity testing as well as the analytical method development and validation at CRL. The standardization is based on critical defined questions and corresponding pre-defined decisions to divert the study towards the correct study set-up. It includes pre-defined moments, where both aquatic toxicity and analytical chemistry experts discuss the challenges from both perspectives including the opportunities from both fields.

Typical examples of difficult test chemicals will be presented regarding the challenges with respect to poor water solubility, stability, or multi-component composition of the test chemical. The structured and standardized approach resulted in a robust and correct study set-up of difficult test chemicals and will be used for future assessments.

### **3.16.P-Th342 The development of salinisation in German waterways from 2000 to 2022**

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In Germany and across most parts of Europe, especially in the non-Mediterranean area, little is known about the extent of freshwater salinisation. Driven by natural processes such as geological weathering, atmospheric salt deposition and saltwater intrusion, as well as anthropogenic activities like mining, irrigating, farming, excessive land use, road de-icing and waste water discharging, overall salinity in German waterways is expected to increase with global warming. Higher temperatures and prolonged and fortified droughts with periods of high evaporation are predicted, leading to higher salt loads, lower water levels and a reduction of the dilution capacity in rivers, especially in the warm summer months. This is problematic as higher ion concentrations cause osmotic stress for freshwater organisms, loss in species richness and reductions in ecosystem functioning inducing high environmental, recreational and economic costs.

To prevent catastrophes like the mass fish die-off in the river Oder in 2022 and a reduction in biodiversity, a better understanding on the state of freshwater salinisation, its development and sources is crucial. So, and to identify and assess the contribution of different salinisation drivers, and to extrapolate to future implications, we compiled an extensive data set from routine state monitoring programs across Germany, covering the years 2000 to 2022. Using this dataset, we plan on using time series analysis to explore the spatial distribution and temporal development of salinity parameters, such as electrical conductivity and salt ion concentrations, and to correlate these to potential freshwater salinization drivers.

Using this data, we already generated the first view on the current state of freshwater salinisation in Germany and its development since 2000, which indicates an increasing trend, accounting for seasonal fluctuations. With a better understanding on the contribution of different salinisation drivers, effective regulations and thresholds could then be established.

### **3.16.P-Th343 Monitoring of seven N-nitrosamines in raw and cooked Korean food by UPLC-APCI-MS/MS**

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N-nitrosamines (NAs), potential carcinogens, can be formed through environmental pollution, additives, and the use of pesticides and fertilizers. NAs, compounds in which nitrogen (N) and oxygen (O) are bonded to an amine group (-NH<sub>2</sub>), can be generated through interactions with contaminants in soil, water, and air, as well as during the cooking process. Therefore, this study aims to explore the differences of NAs levels between raw and cooked Korean foods.

The target NAs for analysis include NDMA, NDEA, NMEA, NDBA, NPIP, NMOR, and NPYR. The total number of samples is 1000, selected from agricultural, livestock, fishery, and processed products based on their intake frequency, consumption, and fat content. This selection included 307 raw foods and 693 cooked foods by 18 representative cooking methods according to the Korean National Health and Nutrition Examination Survey. Sample preparation for the analysis of the seven NAs in food was performed using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method, followed by instrumental analysis using UPLC-APCI-MS/MS.

Total NAs in food were detected at a rate of 58%, with NPYR showing a detection rate of 27% and a maximum concentration of 113 ng/g. Among food categories, agricultural products exhibited the highest detection rate at 61%. In cooked food, the detection rate of total NAs was 54%. Among them, NMOR showed the highest detection rate at 27%, and NDMA exhibited the highest concentration at 150 ng/g. Depending on the cooking method, dry heat cooking led to an increase in NAs levels, with the highest increase observed during direct flame roasting. Conversely, there was a tendency to decrease during moist heat cooking.

This study confirms the diverse detection of NAs in food and observes a wide range of concentrations depending on the cooking method. Therefore, continuous monitoring studies are necessary.

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### 3.17 New Perspectives and Developments in Chemical (Bio)Degradation and Persistence Assessment

**3.17.T-01 Impacts of Sample Storage and Reference Compounds in the OECD 309 Surface Water Mineralisation Test**  
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Biodegradation is an important process for the removal of several environmental organic contaminants and is a major determinant of both chemical- persistence and -exposure, and standardised laboratory tests have been developed for assessing persistence. The OECD 309 method is a simulation test for aerobic biodegradation of chemicals in surface waters and is the preferred method for generating data for persistence assessment under EU REACH. Although the guideline defines several parameters, there are some challenges and uncertainties related to the recommended test requirements which are likely to lead to variation in observed degradation.

In this ongoing work, the OECD 309 Surface Water Mineralisation Test has been investigated by providing an in-depth assessment of inoculum quality and viability; identifying and validating more relevant reference substances that better reflect the vital status and composition of the inoculum; and providing a data-based evaluation of the test robustness and applicability at the 12°C test temperature. By using a suite of quantitative and qualitative tests (as MPN/CFU, respirometric analysis, 16S rDNA microbiome analyses), the data obtained has provided a basis for suggesting improved guidance on surface water sample collection, storage, and treatment. Further, it has been suggested that the currently recommended reference compounds (sodium benzoate and aniline) do not provide good indicators of inoculum performance due to their high biodegradability, and this ongoing Cefic-LRI ECO55 project has therefore aimed at identifying potential new reference substances with a slower/less easily degradation. Based on an initial screening program, three compounds have been selected for further analyses, namely aniline, Caffeine and 2,4-dichlorophenoxyacetic acid. A pre-test to check varying environmental and laboratory conditions have been performed.

The data obtained forms a basis for providing improved guidance on surface water sample collection, storage, and treatment. An interlaboratory ring trial is initiated by the end of 2023 to validate the proposed candidates for use as reference substances under OECD 309. Together the work is intended to support an update to the OECD 309 Test Guideline, to improve its robustness and support reaching reliable persistence conclusions in regulatory processes.

**3.17.T-02 Comprehensive and Non-target Persistence Testing of Chemicals Discharged from Offshore Oil Platforms**  
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In 2018 more than 300 million m<sup>3</sup> of produced water were discharged from offshore oil and gas production to the North Sea. Produced water contains a complex mixture of petroleum hydrocarbons and other chemicals added during production. It is important to avoid discharges that contain persistent chemicals, as concentrations in the environment can otherwise increase over time and have irreversible consequences. It is urgently needed to improve the environmental relevance of biodegradation testing as well as the efficiency in data generation for persistence assessment of chemicals. This study aims to combine environmentally relevant biodegradation tests with advanced analytical methods to reveal persistent chemicals in complex oil platform discharges. Two experiments were conducted, where produced water and seawater were sampled at offshore oil platforms, transported to shore by helicopter and experiments were started within 48h. Test systems were prepared by diluting produced water in seawater (biotic test systems) or in artificial seawater (abiotic test systems). Dilution ratios tested ranged from 1:20 to 1:500. The test systems were set up in closed 20 mL headspace vials. For one experiment, additional test systems were prepared for studying the inhibition of the inoculum by the produced water through measurements of bacteria cell count. The test systems were incubated for 60 days at 9-10°C. Test systems were analyzed by SPME GC-MS and SPE LC-HRMS, and each analysis included triplicate biotic and abiotic test systems. Persistence/biodegradation were evaluated based on peak

area ratios (biotic/abiotic). The GC-MS analysis of experiment 1 (1:200 dilution) showed that 96% of 163 chemicals were more than 50% biodegraded on day 60, including all major peaks. Only 7 chemicals were found to be persistent, and a tentative structural annotation was made for 2 of these. SPE LC-HRMS analysis was applied to a limited number of test systems within the same experiment, and allowed the determination of biodegradability/persistence for additionally 733 features. However, these tests were run at 1:60 dilution, where we observed moderate inhibition of the inoculum. Experiment 2 included tests on produced water from two oil platforms, higher dilutions and a more elaborate non-targeted SPE LC-HRMS analysis. The approach can be used for revealing persistent chemicals in complex discharges, in order to improve treatment or substitute production chemicals.

### **3.17.T-03 Applicability of screening and simulation studies in polymer biodegradation assessments**

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As interest continues in assessing existing and developing biodegradable polymers, there is a need to understand the ability of screening and simulation studies to be used for biodegradation assessments. The standard test methods were developed for and have been largely applied to single constituent, small molecular weight (MW) molecules and their applicability to assess complex multi-constituent polymeric mixtures needs to be evaluated. This research evaluated the biodegradation rate and extent of different polymers in standardized and modified screening studies with activated sludge and river water used as inocula and in wastewater treatment plant simulation studies. Polymers evaluated covered different monomeric starting materials, different functionalization levels, and different molecular weights which are all components known to impact biodegradation. OECD 301B Ready Biodegradation Studies and OECD 302B Zahn-Wellens Studies were conducted using activated sludge as inocula, biodegradation tests using river water as inocula were conducted in a respirometric system following CO<sub>2</sub> evolution as the analytical endpoint, and OECD 303A Simulation Test for Aerobic Sewage Treatment studies were conducted following DOC as the main analytical endpoint for evaluation. Mineralization extent was similar between OECD 301B and 302B studies but time to reach complete mineralization was faster in OECD 302B studies. Mineralization extent in respirometry screening studies with activated sludge inocula was predictive of extent in river water studies but rates were slower in river water studies likely due to lower microbial counts in the test system. The high mineralization quantified in screening studies using activated sludge as inocula was predictive of high removal via biodegradation in wastewater treatment plant simulation studies.

### **3.17.T-04 Persistence assessment in the regulatory assessment and management of chemicals**

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Introduction: Chemical's persistence is a key property heavily influencing or even driving its hazard, exposure or risks. Cousins *et al.* proposed to consider high persistence alone as a major cause of concern [1]. Persistence of chemicals is assessed using degradation rates and degradation half-lives derived usually from laboratory-based simulation degradation tests. Ready biodegradability methods are stringent screening tests and a positive result in such test can be considered indicative of rapid and ultimate degradation in most environments. Simulation tests are relatively expensive and sometimes challenging to conduct. Therefore, use of alternative approaches (e.g. models and/or read-across/grouping approaches etc.) is of high importance.

In ECHA Guidance [2] it is noted that models for predicting ready biodegradation are normally not yet sufficiently accurate to predict rapid degradation. ECHA is aiming to analyse if currently available models (e.g. BIOWIN and Vega) are able to accurately predict the outcome of newly generated reliable experimental data on ready biodegradability.

To speed up the identification of chemicals that need regulatory action, ECHA addresses groups of structurally related substances together. This is done via the assessment of regulatory needs (ARN) which is an iterative and informal screening process, linking the REACH and CLP regulatory processes to enable faster regulatory risk management.

Discussion and conclusions: ECHA is aiming to share results of an analysis conducted to compare results of predictions for ready biodegradability with results of standard experimental ready biodegradability studies requested under dossier evaluation process of REACH Regulation. Furthermore, work on groups of substances with achievements so far in respect of identification of PBT and PMT candidates will be presented. This will include an overview of groups with inconclusive or potential PBT/vPvB/PMT/vPvM hazard identified during ARN screening with a focus on groups containing UVCB substances and how use of grouping and screening information, including QSAR predictions, may support the ARN process.

#### References

- [1] Cousins IT, Ng CA, Wang Z, Scheringer M. Why is high persistence alone a major cause of concern? *Environ. Sci.: Processes Impacts*, 2019, 21, 781-792.
- [2] ECHA Guidances on Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7b. Version 4.0, June 2017.

### **3.17.P New Perspectives and Developments in Chemical (Bio)Degradation and Persistence Assessment**



### 3.17.P-Tu280 Accelerating the Invisible: Unveiling the Impact of Suspended Sediment Concentrations on Biodegradation of Organic Chemicals in Water-Sediment Suspension Systems

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Determining biodegradation is the core of regulatory assessment of chemical persistence. The OECD 309 test is widely used in regulation as a higher tier test for measuring biodegradation in surface water. However, the lack of a clearly defined sediment concentration in the OECD 309 suspension test may cause variability in biodegradation studies. To address this question, the influence of sediment concentrations on biodegradation was investigated using a modified OECD 309 suspension test. The biodegradation kinetics of 129 chemicals were studied at six sediment concentrations, spanning from 0 to 125 g L<sup>-1</sup>, over a 13-day incubation period. A positive linear correlation between the biodegradation rate constant, *k*, and sediment concentration was observed for many chemicals. The strong influence of sediment concentration on *k* indicates that much of the degrading capacity in the suspension test is associated with the sediment. The results indicate that it may be necessary to specify a fixed sediment concentration in the OECD 309 suspension test or find a way to normalize the test results for the effect of sediment concentration. Additionally, the linear relationship demonstrated in our study may be useful for extrapolating biodegradation rate constants obtained from laboratory studies to field conditions.

### 3.17.P-Tu281 Extending the applicability domain of persistence testing of organic chemicals in soil to dissimilar bioavailability scenarios

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Microbial degradation is often the main dissipation route in persistence tests and it operates preferentially on the most bioavailable chemicals, from there the necessity of connect bioavailability to persistence endpoints. Although bioavailability is still unexisting in persistence standardized tests, it is possible to incorporate it into the current OECD persistence simulation tests, therefore providing more realism than that provided by total concentrations of the parent chemical only. With this aim, bioavailability measurements were integrated in persistence testing of organic compounds in situations of partial biodegradation, i.e., not leading to mineralization, and *a-priori* dissimilar bioavailability. In this integration, two methods were used, the OECD 307 guideline for degradation in soil and the ISO method 16751:2020 for bioavailability assessments through Tenax extraction during 20 h. This approach was applied to two <sup>14</sup>C-labelled organic compounds (pyrene and carbamazepine, with Log K<sub>ow</sub> of, respectively, 4.8 and 2.7), and soil and compost-amended soil as dissimilar bioavailability scenarios. With the objective of increasing the sorption of organic pollutants and reducing their bioavailability, traditional compost and co-composted biochar were used as soil amendments. For the two compounds, it was possible to determine the percentage distribution of the parent compound and the metabolites in each phase of Tenax extraction (i.e., soil, water and Tenax) before and after the OECD 307 incubation test. In this way, major bioavailability changes were observed during persistence tests that gave similar dissipation losses of the total parent chemical. With pyrene, the transformation led to lowering the risk due to ageing and the formation of nondesorbable metabolites, whereas with carbamazepine, the remaining parent chemical desorbed more slowly (thus allowing Tenax to act as a perfect sink), but the metabolites showed a high mobility in water, therefore contributing to risk. Polar carbamazepine metabolites remained in the water phase, whereas Tenax adsorbed preferentially the more hydrophobic transformation products and the parent molecule. For both compounds, the bioavailability decreased in amended soils and for carbamazepine the strongest bioavailability reduction was measured with a co-composted biochar amended soil (70 % compost with 30 % biochar).

### 3.17.P-Tu282 Prioritisation of known contaminants in drinking water resources for a biodegradation simulation test according to OECD Test No. 309: Aerobic mineralisation in surface water

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In 2023, the German Environment Agency (UBA) published a broad literature search (55 studies between 2000 and 2019) listing 1289 chemicals that have been detected to date in the six water matrices wastewater treatment plant effluent, surface water, bank filtration water, groundwater, raw water and drinking water (FKZ 3719654080, UBA TEXTE 20/2023). However, for 47% of the 509 REACH-registered substances detected, no conclusive assessment of persistence in the aquatic environment is available. The need for radioactive labelling of the test substance is generally used as main reason why an OECD simulation test is difficult and expensive. However, under specific conditions, a valid and reliable OECD TG 309 test results can be carried out by any water laboratory without an isotope laboratory. In Germany and Europe for example the large to medium size drinking water suppliers and the water monitoring authorities have such laboratories.

Non-radiolabelled test substances which dissipate from the test vessel are not suitable for a "cold" OECD TG 309 as this would lead to an invalid and unreliable result. We assessed a total of 1271 chemical structures known to contaminate different water matrices. As a result, we de-prioritised 222 chemicals structures based on a high potential for adsorption and/or a high potential for volatilisation and we de-prioritised further 158 based on consistent information on an available potential for degradation.

We prioritised the remaining 891 known contaminants as they are "easy to test chemicals" with a high risk of lack of biodegradation potential and consequently they are suitable for a "cold" OECD TG 309 tests system. If such a test result is

without uncertainty, also the "cold" OECD TG 309 fulfils the high quality and reliability requirements of e.g. the ECHA PBT EG and the MSC and can then be used to derive a half-life for the conclusive persistence assessment.

The German Environment Agency (UBA) expects an increasing demand from drinking water suppliers and water monitoring authorities to carry out a "cold" OECD TG 309 in their own analytical laboratories for contaminants in their local watershed. Our work will support this demand and also provides recommendations how to reduce the variability between test vessels, minimising uncertainty and thus strengthening the overall validity of a "cold" OECD TG 309 test result.

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### **3.17.P-Tu283 Application of (enhanced) ready biodegradability tests in persistency evaluation**

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(Enhanced) ready biodegradability tests (eRBTs) are used to show that a substance is non-persistent, when the pass level of 60% is reached within 60 days. However, negative results in these tests are inconclusive and require the application of complex OECD simulation tests. The applicability of inherent tests in persistence evaluation seems being limited, because of the pass level must be reached within 14 days. Recently, the Microplastic Regulation (EC) No 1907/2006 established a similar testing scheme, ready tests, eRBTs and inherent tests (OECD 302 C), but also included several tests specifically designed for polymers for different compartments while allowing test durations up to 6 months.

In a practical testing programme 5 compounds (non- polymers) with controversial degradation data have been tested in 4 ready test series including prolongation to 60 days and use of different inocula (activated sludge, final effluent from a STP), flask sizes, and endpoints (CO<sub>2</sub>, O<sub>2</sub>, DOC). Two of them, the drug ibuprofen and the intermediate 4-fluorophenol were readily biodegradable. The pesticide synergist piperonylbutoxide and the antioxidant octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate achieved 20%–50% (=“potentially P”) while the cosmetic ingredient cis-13-docosenonamide (Erucamide) showed degradation extents between 21%–64% after 60 days.

Surprisingly, diethylene glycol reached the pass level of 60% mineralization within 28 days in all test series and thus proved to be a suitable reference substance for eRBTs next to inherent tests. In this context also the role of eRBTs in relation to inherent biodegradation screening tests in the context of persistence evaluation needs to be clarified.

### **3.17.P-Tu284 Accumulating Knowledge of Biodegradability Weight-of-Evidence Approach for Persistent Assessment of Difficult Substances**

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Appropriate management of persistent substances is one of the crucial issues for achieving a sustainable circular economy. However, evaluating the degradation half-life of substances through simulation tests to examine the persistence criteria is cumbersome, and it is important to assess non-persistence based on other test information for efficient evaluation. Although the European Chemicals Agency (ECHA) has published guidance for persistence, bioaccumulation, and toxicity (PBT) assessment (Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment. (version 3.0)), it does not indicate which test results, except for the (enhanced) ready biodegradability test and inherent biodegradability test, would satisfy the weight-of-evidence approach. However, substances with specific properties such as being poorly water soluble, volatile, adsorptive, unstable, or toxic to inoculum, often called difficult substances, present difficulties in evaluation of their intrinsic biodegradability by these studies, so accumulating knowledge through non-guideline tests is desirable.

In this study, we conducted several biodegradability studies for poorly water soluble and cationic toxic substances. To evaluate the intrinsic biodegradability, we performed testing at low concentrations, with additives, and in river water.

For poorly water soluble substances, the degree of degradation varied depending on the length of the alkyl chain and several substances showed greater than 50% degradation at 40 days in low-concentration tests conducted at 5 mg ThOD/L with activated sludge. Also, degradation progressed quickly with aeration in the degradation test using river water. On the other hand, no significant improvement in degradation rate was observed with the dispersants. For cationic toxic substances, the addition of linear alkylbenzene sulfonate (LAS) as an additive accelerated degradation in the test with activated sludge, and degradation tests using river water also confirmed that degradation progressed quickly.

Based on these studies, both substances were considered to be non-persistent as a result of the weight-of-evidence approach. In this presentation, we will explain the non-guideline tests that we conducted and discuss which non-guideline study results are appropriate for a weight-of-evidence decision for difficult substances.

### **3.17.P-Tu285 Preserving the Biotransformation Potential of Activated Sludge in Time: Towards Reproducible Incubation Experiments for Persistence Assessment**

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Due to their widespread presence, (pseudo-)persistence, and potential ecotoxicological effects, micropollutants pose a growing environmental concern. Environmental persistence, as one potential key hazard, is typically assessed for environmental compartments such as water, soil and sediments. Biotransformation assays conducted in activated sludge from wastewater treatment plants offer various benefits over soil and sediments, as activated sludge is easily manageable and readily available. Additionally, the high microbial density enables a short experimental duration of 72 hours, and rate constants determined in activated sludge experiments have been shown to be reasonably predictive of soil half-lives. These points imply that conducting biotransformation experiments with activated sludge could serve as a valuable foundation for developing high-throughput persistence tests used for screening purposes, e.g., in a benign-by-design framework. Despite standardized procedures under the REACH regulation, screening tests in activated sludge though face challenges due to low reproducibility linked to inocula diversity.

In this study, we developed protocols for the preservation of activated sludge microbial communities using lyophilization or cryopreservation. Their performance in preserving the biotransformation potential was evaluated on forty representative micropollutants in lab-scale batch assays lasting 72 hours, with fresh activated sludge as reference. Lyophilization was the least representative preservation method, with over 85 % of micropollutants showing significantly slower or faster biotransformation compared to fresh activated sludge. Cryopreservation using either DMSO or glycerol was more effective with approximately 40 % of micropollutants exhibiting significantly different biotransformation kinetics than in the fresh matrix. We also explored the impact of the storage time (two weeks vs. four month) as well as the effect of using artificial supernatant instead of preserved native supernatant. We examined shifts in community composition based on amplicon sequencing data. Our results indicate that cryopreservation can effectively preserve the biotransformation potential of activated sludge, improving long-term reproducibility in persistence assessments. This enhances reliability for regulatory hazard assessments and biotransformation screening in the design of novel chemicals.

### **3.17.P-Tu286 Microbial community analyses of a ring test for a new marine biodegradation test for persistence screening (MaP test)**

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A newly developed screening test for substances in the marine environment, the Marine Persistence (MaP) test, has recently been accepted as a method under the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR). The MaP test incorporates increased prokaryotic cell numbers with the aim to better represent the microbial (prokaryotic) diversity inherent in the sampled environments and runs beyond 60 days, which is the half-life threshold for persistence in the marine environment. The MaP test underwent a ring test comprising 13 laboratories and was found to provide a more reliable and less variable characterization of the biodegradation behavior of five reference chemicals (sodium benzoate, triethanolamine (TEA), 4-nitrophenol (4-NP), anionic polyacrylamide, pentachlorophenol), than the current marine biodegradation screening test, OECD 306, with respect to REACH and OSPAR persistence thresholds.

Microbial analyses demonstrated that prokaryotic communities in replicate samples from the same location were significantly more similar than those between different locations. An average dissimilarity of 91% between the prokaryotic communities sampled from the different locations used in the study suggests that prokaryotic communities in the marine environment are very diverse. There was a positive relationship between total cell counts and biodegradation potential. Half the samples in which the prokaryotic cells were concentrated were also associated with an increase in the observed number of prokaryotic taxa ("species"). However, there was no statistically significant relationship between total cell counts and prokaryotic diversity indices: the greatest rates and extents of degradation were not necessarily observed for samples of prokaryotic communities with the highest cell concentrations or diversity indices. The probability of biodegradation of TEA and 4-NP was significantly negatively correlated with salinity, which was significantly correlated with the depth and distance from shore that the samples were sourced (Pearson's correlation,  $P < 0.01$ ). Those sources which resulted in the biodegradation of TEA and 4-NP were associated with genera that were either differentially over- or under-represented in the prokaryotic communities studied compared to those that showed no biodegradation. These findings suggest that prokaryotic community composition rather than prokaryotic diversity *per se* is important in driving biodegradation.

### **3.17.P-Tu287 New Approaches to Persistence Testing With Increased Cell Number Using Tangential Flow Filtration.**

*Katie Endersby, Dave Gore and Chris Finnegan, Safety and Environmental Assurance Centre (SEAC), Unilever, United Kingdom*

Assessment of a chemical's persistence is fundamental to understanding its environmental fate, exposure and ultimately for determining environmental risk. It is one of the key criteria used for the regulation of organic chemicals. Evidence for determining a chemical's potential persistence is primarily obtained from the understanding of its biodegradability in relevant test compartments, derived from biodegradation screening and simulation tests. Current testing frameworks, including OECD Testing Guidelines, are recognised as having limitations with difficult to assess chemicals such as large, polar, or poorly soluble materials. Testing is often not reflective of real, variable environmental conditions, with little consideration for the microbial inoculum captured within tests or its influence on test outcomes. To support better understanding of the potential persistence of chemicals, new methods have been proposed to facilitate more consistent testing for hard to assess chemicals, including increasing the concentration of microbial inoculum in tests.

Tangential flow filtration (TFF) techniques can be adopted for use with aqueous environmental samples. The approach can negate inconsistencies in biodegradation often seen in testing using environmental samples with low microbial biomass, such as freshwater, by increasing the probability that the chemical tested will encounter a potential microbial degrader within the test media. In addition to allowing a larger, more representative proportion of a microbial community to be captured within the test, predictions of persistence can be carried out within shorter testing timeframes than traditional tests, with increased bacterial number often facilitating a shorter lag period in detectable chemical degradation.

Here we present how new TFF approaches can be integrated with current screening methods, i.e., OECD Test Guideline 301F, by using samples of increased microbial number, and including measurements of bacterial enumeration in testing protocols, to better ascertain a chemical's degradation kinetics. Microbial -omics techniques can subsequently be utilized to investigate the lack of knowledge on the influence and dynamics of microbial communities in test systems, to characterize the catabolic potential of real environments used in testing, and to generate data that can be used to support new in-silico approaches to predicting persistence of chemicals; ultimately improving assessments of environmental risk.

### **3.17.P-Tu288 Chemical Biodegradability and Persistence Assessment**

*Kirit Wadhia, Environmental, NOV Inc, United Kingdom*

In consideration of ecotoxicological perspective regarding the impact of chemicals in the environment, indubitably it is important to ascertain the chemicals' persistence. Thus, a significant criterion is the rate of degradation in the environment and of particular value tests that can expeditiously screen out chemicals which are prone to degradation in diverse environments.

Biodegradation determination of chemicals is critical as it provides valuable insight of exposure and subsequently the long-term adverse effects on biota and implications for mankind (OECD, 2006).

Biodegradation screening tests (BSTs) have been developed and approved by the Organisation for Economic Co-operation and Development (OECD). BSTs are often a vital integral component of regulatory frameworks; OSPAR Agreement (2012-05) dealing with Harmonised Chemical Notification Format (HOCNF) is a casing point. Its remit is the management of offshore exploration and production activities in the OSPAR maritime area. Their prominence in this context has been due to the associated relative low cost and presumed ease of implementation and interpretation.

However, biodegradation evaluation intrinsically is complex and unpredictable. Whereas the intended initial purpose of the BSTs was to screen out chemicals which easily degrade, they have not evolved at the same rate as regulatory concerns, which now place an increased emphasis on environmental persistence.

Since the suitability of BSTs, for example OECD 306, is not deemed to be robust to identify persistent chemicals, projects to modify protocol have been instigated and proposal submitted to OECD for a new test guideline for a marine biodegradation screening test for chemical persistence assessment (MaP test).

The MaP test incorporates increased bacterial cell numbers and uses manometric respirometers to run for 60 days or more. It has undergone ring-testing with the participation of 13 laboratories in 7 countries.

Does it have application in marine regulatory frameworks and conventions

Reference:

OECD, 2006. OECD Guidelines for the Testing of Chemicals: Revised Introduction to the OECD Guidelines for Testing of Chemicals, Section 3, OECD Guideline for Testing of Chemicals (Paris).

OSPAR (2012), Guidelines in support of Recommendation 2012/5 for a Risk-based Approach to the Management of Produced Water Discharges from Offshore Installations. Agreement: 2012-7. London, UK.

### **3.17.P-Tu289 "Characterization of Industrial Wastewater Samples: Application of a Degradation Assay and Bioassays"**

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Industrial and commercial wastewaters often contain unknown components and mixtures of substances. Some of them can be of concern, but they can only be identified to a limited extent and sometimes great expense by chemical analysis. Thus, it is of great interest to develop practical alternative approaches to characterize industrial wastewater streams without affecting trade secrets. Flagging problematic streams can foster the establishment of suitable disposal routes or wastewater pretreatment steps in the future to reduce the input of non-degradable problematic substances in wastewater treatment plants (WWTP) and latter surface water systems.

Bioassays do provide an interesting possibility for the characterization of industrial wastewater, but to date the potential has rarely been exploited in Switzerland, as there is no legal basis for the use of biological test methods and there is a lack of

experience among companies and authorities. The ABIScreen ("Abbautest Biotest Industrieabwasser Screening") screening approach developed at University of Applied Sciences and Arts Northwestern Switzerland (FHNW) combines a newly established time-efficient degradation test, simulating the WWTP degradation, and a bioassay battery to characterize industrial wastewater to close this gap.

The alternative inherent degradation test ("Alternativer Inhärenter Abbautest AIA") determines the rate of biodegradation and the refractory organic load of an (industrial) wastewater sample four times faster than the commonly used Zahn-Wellens test and also provides information on nitrifier inhibition. The ecotoxicological bioassay battery is used to estimate the toxic potential of the non-biodegradable substances in the sample. The test organisms used are daphnia, algae and luminescent bacteria as well as a mutagenicity test to visualize the effects of all components contained in a sample, regardless of their chemical identification status.

ABIScreen can be applied to identify problematic industrial wastewater with non-biodegradable substances and increased toxic potential. The first large-scale ABIScreen campaign is currently underway in Switzerland to test industrial wastewater from various sectors with ABIScreen and to compile a broad collection of data. The data basis will be used to develop threshold values for the classification of toxicity values on the one hand and industry-specific adaptations of the screening tool on the other.

### **3.17.P-Tu290 Ultimately Biodegradable, or Not Ultimately Biodegradable - That is the Question!**

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Ultimate biodegradation signifies the ability of bacteria to mineralize organic chemicals by converting the carbon skeleton into CO<sub>2</sub>. To be considered ultimately biodegradable, a substance typically needs to reach the pass level of  $\geq 60\%$  within a specified timeframe according to international test guidelines such as OECD 301. These tests are stringent and a negative result does not necessarily mean a chemical will not degrade in the environment.

Whether a substance is ultimately biodegradable or not can be studied with more targeted studies, e.g. over a more prolonged time window or using an inoculum after an enrichment culture or using specific isolated bacteria. Such tests do not indicate biodegradability under OECD accepted conditions, but they indicate whether the initial sewage sludge contains bacteria able to express the relevant enzymes to ultimately degrade a particular substrate. Such data may add to a weight-of-evidence assessment on persistency.

Here, we present our work on 2-cyclohexylidene-2-phenylacetonitrile (Peonile). This chemical was selected because it is composed of biodegradable substructures (mainly a phenyl and a cyclohexane-ring) yet only partial mineralisation is observed in OECD 301F tests. Further, the molecule lacks typical non-biodegradable features, such as extensive alkyl-branching or quaternary carbon atoms.

We tested Peonile for mineralisation by an adapted inoculum after three 28-days cycles of enrichment. This inoculum was able to mineralise Peonile with a biphasic curve. Isolation of bacteria from this inoculum first led to isolates able to degrade the phenyl ring of Peonile generating the stable metabolite, 2-cyano-2-cyclohexylideneacetic acid. However, isolation of further bacteria at the end of a mineralisation experiment conducted with this adapted inoculum led to a bacterial strain able to degrade the metabolite. A mixture of the two bacteria types was then tested under OECD 301D and 301F incubation conditions and shown to mineralise Peonile.

The need for two separate bacteria for mineralisation explains the biphasic curve observed in the mineralisation by the adapted sludge, yet it indicates that Peonile is ultimately biodegradable by bacteria originally present in sewage sludge. It appears, that under the dilute conditions of the standard OECD tests, the bacteria performing the second step do not get sufficiently enriched to allow for this mineralisation to occur within the time window of OECD screening tests.

### **3.17.P-Tu291 Biodegradable or not? Developing a standardized international approach to assessing the biodegradability of cosmetic formulations**

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Consumer expectations and demands for environmentally benign, biodegradable product formulations are increasing rapidly. Although biodegradability assessment of cosmetic formulations is included in various certification and labelling programs and environmental/sustainability scoring tools, so far, no clear guidance is available to address how to consistently assess the

biodegradability of cosmetic formulations. Therefore, the global non-for-profit science organization International Collaboration on Cosmetics Safety (ICCS) is undertaking development of an internationally validated process setting out standardized approaches for evaluating the biodegradability of cosmetic ingredients including how to calculate the biodegradability of product formulations. To support the development of a standardized approach, a critical review of regulatory positions, existing criteria, and standards specifically for assessing the biodegradability of ingredients, formulations and chemical mixtures is being performed and will be presented in this poster. The review includes cosmetics, personal care, and homecare products. Based on the results of the review, suggestions regarding international standard development will be mapped and a roadmap for gaining acceptance of a possible international standard will be highlighted.

### **3.17.P-Tu292 Assessment on the Degradability of Emerging Contaminants in Freshwater and Marine Sediments**

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Contamination of aquatic systems by newly detected chemicals, such as many pharmaceuticals and personal care products, represents a rising concern. Many organic contaminants are not persistent *per se* but their occurrence is owing to their continuous release into the aquatic systems. This encourages the need to assess the environmental behavior and to investigate the microbial degradation processes involved which could remove or reduce their concentration in the environment. Among the different environmental compartments involved are sediments, which, due to the hydrophobic nature of many organic contaminants, tend to accumulate significant fractions of these chemicals, acting as natural sinks.

In this study, we have conducted a 100-day incubation experiment to determine the partitioning and microbial degradation of 5 selected pollutants in two systems: a fresh water- river sediment microcosm and a seawater- marine sediment microcosm, where microbial communities are expected to be significantly different. The five chosen contaminants were: the insect repellent N,N-diethyl-meta-toluamide, the organophosphorus flame retardant tris(2-chloroethyl) phosphate, the fragrance OTNE (Iso E Super) and two UV filters, octocrylene and benzophenone-3 (BP-3). Samples were taken at regular intervals and contaminants extracted by pressurized liquid extraction with in-cell clean-up (in-cell PLE) for the particulate fraction and by stir-bar-sorptive-extraction (SBSE) for the aqueous fraction. Separation and quantification of target analytes were performed on a GC-APGC-Q-ToF-MS instrument (Bruker Impact II) and the processing was performed using TASQ software 2022b.

Sorption coefficients and degradation kinetics have been determined for the aforementioned target pollutants. The fraction of chemicals accumulated in sediments varied accordingly with the octanol-water partition coefficient of the chemicals, from lowest (e.g., DEET) to highest (e.g., OC). The bioavailability of each contaminant had a significant impact on the degradation and speed, with BP-3 showing the highest removal (>90%). Differences between fresh and marine microcosms were also observed, indicating that variability in microbial communities can heavily impact the degradation process from one depositional environment to another.

Köchling, T., et al(2011). Microbial community composition of anoxic marine sediments in the Bay of Cádiz (Spain). *International Microbiology*, 14(3), 143-154.

### **3.17.P-Tu293 Variability of Biodegradation Rates in Rivers from Different Regions of Europe**

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Contaminant attenuation varies considerably between rivers, and this has been attributed in part to spatial differences in biodegradation. However, the variability of biodegradation rates between aquatic systems has not been studied for a large number of chemicals. To study systems with broad climatic and geographic variability, we collected samples from 19 different river segments in the Mediterranean (Spain and Greece), alpine (Switzerland), central plain (Germany), and boreal (Sweden) regions of Europe during summer and early autumn (20.5 ± 3.5 °C). In each country, we sampled pristine river segments as well as segments strongly impacted by urban wastewater effluent. We performed a modified OECD 309 test with water and sediment from each river segment. An aqueous mixture of 129 compounds was spiked to a concentration of 1 µg L<sup>-1</sup> each and their dissipation was followed over a 10-day incubation. Significant differences in biodegradation rates (ANOVA, *P* < 0.05) between the 19 river segments were observed for 98% of 97 quantified compounds. We found that the spatial variation in biodegradation rates between pristine river segments across countries was smaller than the variation observed between contaminated river segments. Additionally, we identified regional specificity in the biodegradation rates of certain compounds that share specific functional groups.

### **3.17.P-Tu294 Field-to-lab Microbial Profile of Batch Incubation Experiments for Biodegradation Testing**

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Biodegradation tests are essential for assessing organic chemical persistence in the environment. The OECD 309 test is a highly recognized method for measuring biodegradation rates of organic test substances in aerobic natural surface water

through pelagic or suspended sediment batch incubations. Although it is a highly recommended biodegradation test, inconsistencies and variable results are still some of the significant issues that need further assessment. These may result from the guideline's lack of consideration of both the biological and ecological complexities in the environmental sample. Microbial communities are the key drivers of environmental degradation and are naturally subjected to temporal and spatial variation. However, we still lack a fundamental understanding of the microbial dynamics of environmental samples in simulated tests. This raises concerns about whether the OECD 309 test provides environmentally relevant estimates of biodegradation compared to in situ assessments. Knowing how each step, from field collection to laboratory processing and batch incubation experiments, influences microbial diversity and community composition could provide important insights for the further standardization of simulated biodegradation testing. Here, we profiled the diversity and composition of the sediment microbiome from field collection and sediment processing in the laboratory to the batch incubation experiments. We followed the OECD 309 test guidelines for sediment-water collection and preparation, and sampled a river segment with homogenous environmental conditions. Five sediment cores were collected along a 20-meter transect, and a subsample of these sediments was pooled to represent one homogenized sample. We profiled the microbiome of all six sediment samples from the field, processing in the laboratory after 18 hours, and during the 1<sup>st</sup>, 5<sup>th</sup>, and 10<sup>th</sup>-day batch incubation in a setup spiked with 129 organic compounds. In this study, we present a better understanding of the microbial dynamics of sediment samples and their influence on the OECD 309 test. Our observations will be useful in improving our knowledge of the microbial community response to field and laboratory conditions before and after starting biodegradation tests. This will also provide insights into the environmental relevance of sediment microbiome samples used for standard simulation tests.

### **3.17.P-Tu295 Building Knowledge from Available Degradation Simulation Studies to Improve their Usability**

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Persistence assessments are an increasingly important component in regulatory evaluation and management of industrial chemicals. In recent years there has been a significant increase in requests from the European Chemicals Agency for degradation simulation studies (OECD TG 307, 308, 309) - 50% increase from 2020 to 2021. Additionally, these requests are foreseen to increase even further with the newly added Persistent, Mobile and Toxic and very Persistent and very Mobile hazard classes in the CLP regulation. However, degradation simulation studies are complex, costly and can be technically challenging for a broad range of chemicals. In this context, it is important to ensure that the test produces adequate and relevant results to be used in the Persistence assessment for these regulations. We aim to collect and curate information available from OECD 309 degradation simulation studies from various sources and use necessary meta data to understand which are the most influential factors that may be affecting the results of these tests. The factors considered include substance properties and test system set-up. The ultimate goal is to identify several critical factors which could enable stakeholders to design better studies and guide the Persistence assessments with scientifically robust approaches.

### **3.17.P-Tu296 How do River Channel Geometry and Sediment Calibre Affect the Degradation of Wastewater Pollutants? Insights from a Laboratory Experiment**

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Microbially mediated transformations (e.g. biodegradation and nitrification) are often the most important mechanisms for the removal of wastewater pollutants in rivers. These transformations predominantly take place in fixed biofilms attached to sediment on the channel bed and banks. This means that in-stream transformation rates are likely to be affected by channel geometry which determines the extent of chemical contact with the biofilms. It is hypothesized that microbially mediated transformation rates will be inversely proportional to hydraulic radius (the ratio of channel cross-sectional area to wetted perimeter) – i.e. transformations will be more rapid in wide, shallow channels than in narrow, deep ones. Microbial degradation is also expected to be more rapid in channels with fine bed sediment, compared to coarse sediment because the sediment surface area available for biofilm colonisation per unit bed area is greater. We tested these hypotheses in controlled laboratory experiments by monitoring the nitrification of ammonium and biodegradation of linear alkylbenzene sulphonate over two-week periods in mesocosms with different channel geometries and sediment sizes. Models of chemical transformation which account for lags in microbial development were also developed to describe the observed phenomena. Microbial degradation was inversely proportional to hydraulic radius. The sediment size experiment revealed a more complex set of controls which, in addition to sediment calibre, are likely to include bed permeability which affects exchanges of nutrients for biofilm development and contaminants for processing. This study highlights the need to consider geomorphology in chemical exposure modelling and environmental risk assessments.

### **3.17.P-Tu297 Electron Donor Availability and Biodegradability Dictates Co-metabolic Organic Micropollutant Biodegradation**

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Biodegradation of organic micropollutants is a key process determining fate and supporting attenuation of these compounds in the environment. Typical micropollutant concentrations in the ng/L- µg/L range are too low to support microbial activity as the sole carbon substrate. Instead, biodegradation commonly occurs co-metabolically, thus requiring an additional primary substrate to support metabolic activity. The availability and biodegradability of this primary electron donor therefore dictates microbial activity and consequently micropollutant biodegradation. This study investigated how different electron donors with varying biodegradability affect micropollutant biodegradation in oligotrophic groundwater. We monitored biodegradation of 15 micropollutants in simulated nitrate reducing groundwater systems in upflow columns. We tested both the effect of electron acceptor (oxygen) addition to create (micro)aerobic conditions as well as different electron donor biodegradability (humics, dextran, acetate, and ammonium). Under nitrate reducing conditions, no micropollutant biodegradation was observed regardless of the electron donor added. However, when oxygen was added, 2,4-D and MCPP were biodegraded.

Biodegradation rates were highest under fully aerobic conditions when acetate was added. Micropollutant biodegradation was lower under microaerobic conditions, most likely due to oxygen limitation resulting from competition with the supplemented electron donor for oxygen. We observed that with time, microbial communities grew and adapted, yielding higher biomass densities and higher biodegradation rates. We also examined the development of the microbial community by sequencing a fragment of the 16S rRNA gene. We found that the microbial community composition was overall dictated by electron acceptor and electron donor availability, not micropollutants, supporting our hypothesis that supplementing alternative electron donors is crucial for supporting overall microbial activity and thus micropollutant biodegradation. Our results indicate that the overall richness and diversity of the community was low, which probably limited biodegradation of other micropollutants tested. Overall, this study provides insight on the role of primary electron donors in selecting for biodegradation of micropollutants as secondary substrates, which can be used for assessing micropollutant environmental fate as stimulating biodegradation.

### **3.17.P-Tu298 Challenges with biodegradation simulation testing of difficult substances: a look at volatile hydrophobic substances in OECD 309**

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Higher tier biodegradation simulation tests are used to assess the degradation kinetics and transformation products of substances under realistic environmental conditions. Data from such tests are utilised in persistence assessments, and are information requirements under several chemical regulations in the EU, such as REACH and PPPR. Simulation tests include the OECD test guideline (TG) for testing of chemicals in surface water – the OECD TG 309.

Certain physicochemical properties of substances can have important implications for their behaviour in standardised tests, and the ability to reliably measure the property under investigation. These substances are termed ‘difficult to test’. In the area of biodegradation testing, substances that are both hydrophobic and volatile can be particularly challenging. Test systems may need to be modified in order to account for these challenging substance properties. These modifications may have implications for the reliability and relevance of the data generated. The OECD TG 309 specifies a maximum Henry’s law constant of test substances as part of its applicability (<100 Pa.m<sup>3</sup>/mol). However, testing of substances of greater volatility has previously been requested under REACH.

In this presentation, the assessment of volatile hydrophobic substances under OECD TG 309 will be explored. The concept of OECD TG 309 will be presented, including experimental conditions and boundaries as specified in the guideline, and the data generated. The implications of volatile hydrophobic test substances will be described, in the context of both behaviour of the test substance in the system, as well as guideline validity criteria. Potential adapted and alternative test systems for assessing degradation rates in surface water will be presented and discussed. These will include known examples using passive dosing and solvent carriers. The advantages and disadvantages of these systems will be discussed, as well as recommendations for future research to further develop suitable test systems for assessing the degradation of volatile hydrophobic substances in surface waters.

### **3.17.P-Tu299 Non-Extractable Residues in Persistence Assessment: Effect on the Degradation Half-Life of Chemicals**

*Cindy Michala Jespersen and Stefan Trapp, Technical University of Denmark (DTU), Denmark  
Cindy Jespersen and Stefan Trapp*

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In the European Union, persistence assessment of pesticides and other substances is done in simulation tests in soil, sediment, or surface water (OECD TG 307, 308 and 309), using <sup>14</sup>C-labeled substances. Non-extractable residues (NER) are formed in every degradation test with labeled parent substance. The guidelines for handling these non-extractable residues (NER) in



persistence assessment have recently been changed from more or less disregarding NER to consider all NER as non-degraded unless demonstrated otherwise. In this project our aim was to investigate how calibrated degradation half-lives change with different approaches towards NER. Moreover, we also tested whether the amount of (harmless) biological residues (bioNER) is relevant, and whether it can be estimated by the Microbial Turnover to Biomass (MTB) method. We collected data from 48 degradation tests of 24 compounds and compared the following scenarios: i) parent compound only (former approach used in ECHA and EFSA), ii) parent + total NER (new default approach in ECHA), iii) parent + total NER-MTBbioNER, and iv) parent+NER I (experimental). The degradation half-life (DegT50) was found for all four scenarios using the software tool CAKE (Computer Assisted Kinetic Evaluation). We found that there is a trend in half-lives from shortest to longest: i) parent only, iv) parent+NER I, iii) parent+total NER-MTBbioNER, ii) parent + total NER. As scenario i) is no longer acceptable, the shortest possible half-life following the new guideline can be obtained by quantifying the amount of NER I experimentally (scenario iv). In 2 cases the quantification and subtraction of bioNER using the MTB method changed the outcome of the persistence assessment from persistent in scenario ii) parent+total NER (current mandatory method) to not persistent in scenario iii) parent+total NER-MTBbioNER. In this study we found that the approach towards NER can have large impact on the outcome of the persistence assessment and that experimental characterisation of NER can lower the DegT50 values significantly. As determination of MTBbioNER is a calculation method, it doesn't require any new laboratory tests and can be implemented in the persistence assessment as a cheap method for refining the DegT50.

### **3.17.P-Tu300 The Use of Tritium Labelled Compounds in Environmental Fate Studies: Considerations and Experience** *Katerina Hamnett, Fera Science Ltd., United Kingdom*

Where possible Carbon-14 is the isotope of choice in for environmental fate studies. The positioning of the radiolabel allows investigation of the fate of the test item in environmental matrices. However, in some instances radiolabelling may not be compatible with the synthesis route of the compound. It may therefore not be possible to synthesise the compound of interest in sufficient quantities or at all. In addition, the synthesis of Carbon-14 radiolabelled compounds can be costly and time consuming. As a result of these issues, it is becoming more common for experimental studies to move to non-labelled test items or tritiated test items. A non-labelled test item does not enable the identification of transformation products or the quantification of mass balance within study samples. Hydrogen-3 (tritium) may therefore be a better alternative for use.

The benefits of using tritium include identification of transformation products, quantification of mass balance in samples and due to the positions of the label accurate measurements can be made with much smaller quantities of test item. In addition, there will be more tritium labelled transformation products when compared to compounds containing the Carbon-14 label as tritium compounds are labelled throughout the structure.

However, there are limitations to working with tritium mainly due to the transfer of the of energy to light water. As a result, radiochemical detection alone may not be suitable for quantifying the test item remaining in environmental samples.

This poster will look at how a study can be designed to incorporate both radiochemical and non-radiolabelled (LC-MS/MS) analysis to quantify test item transformation in environmental fate studies. It will incorporate the investigation of tritium labelled test items where carbon labelling is not possible and include confirmatory LC-MS/MS analysis to support findings. In addition, where transfer of the tritium label to water or other products present in environmental samples occurs, a route to provide usable data from these studies using non-radiolabelled confirmatory analysis will be presented.

### **3.17.P-Tu301 Time-dependent overall persistence as a tool in a weight-of evidence for persistence assessment**

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Chemical persistence plays a key role in the determination of environmental exposure making it an important endpoint in risk assessment and regulation. For persistence assessment information is required on the degradation rates in different compartments (soil, water, sediment, air). However, many substances are difficult to test or fall outside the applicability domain of existing guidelines due to their specific characteristics. In addition, degradation rates are prone to wide variability depending on environmental conditions. Finally, evaluating degradation half-lives using a compartment-by-compartment approach is overly simplistic, and neglects dynamic multimedia exchanges and degradation processes that may have an important bearing on the overall persistence of a substance in the environment. Results from multi-media modelling could be used case-by-case in order to evaluate the environmental exposure and compartment(s) of specific concern in a qualitative or semi-quantitative approach. Furthermore, overall persistence (Pov) has been proposed to be a suitable replacement metric for the compartment-specific half-lives in persistence assessment.

The multimedia model MUST (level III and IV) is applied on case studies taking into account different properties (degradation half-lives, volatility, solubility, adsorption), and different mode of entries. In addition, emission scenarios such as emission stop are used to evaluate the impact on the overall persistence and the relevant compartment. Overall persistence is given usually for the steady-state concentration. However, concentration in the different compartments will change over time due to distribution and different degradation rates. For this reason, overall persistence will change over time (Pov(t)) as well. For this reason, overall persistence is calculated for each time point of environmental exposure to illustrate the alteration over time. Such simulations may give additional evidence on the persistence of a substance, improve the robustness of current persistence

assessment methodologies, and the option to use multimedia modelling (overall persistency) in a weight-of-evidence approach for persistence assessment.

### 3.17.P-Tu302 Assessing Chemical Persistence: The Imperative of Looking Within Our Homes

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“Persistence” describes a chemical’s resistance to physical, chemical and biological elimination from the environment. It is a crucial indicator for characterizing the long-term, irreversible exposure risks to humans and wildlife. To date, the frameworks for assessing environmental persistence have exclusively focused on *outdoor settings*, such as freshwater, soil, and sediment. However, chemical usage is not limited to outdoor settings. *Indoor spaces*, such as homes and offices, are also significant sources of chemical release, including substances like flame retardants, plasticizers, indoor pesticides, and liquid crystal monomers (LCMs). Indoor “near-field” contamination has been identified as a major pathway for human exposure to these substances. Notably, these substances can accumulate significantly in the human body without needing to persist in outdoor environmental media or accumulating in food and water sources. However, less attention has been paid to *defining* and *quantifying* the persistence of chemicals in indoor environments. In this presentation, we use 93 LCMs as an example of chemicals originating indoors to compare and contrast their “indoor persistence” and the commonly studied “outdoor persistence”. We use an established and validated model named “PROduction-To-EXposure (PROTEX)”, which is distinguished from other models by its unique ability to integrate the fate of chemicals in nested indoor, urban, and rural environments. Our findings reveal that when outdoor persistence is selected as the point of assessment, <10% of the investigated LCMs are more persistent than known persistent organic pollutants based on the “overall persistence” indicator, and 1/3 to 2/3 of the investigated LCMs meet the criteria for persistence set by the Stockholm Convention based on medium-specific half-life thresholds. However, our analysis demonstrates that most LCMs tend to persist for much longer periods indoors compared to outdoors, mainly due to their low volatility and strong partitioning into indoor surface compartments. Notably, the indoor environment may serve as a reservoir to sustain the long-term regional contamination with LCMs after the cessation of indoor release. This presentation reveals and highlights the importance of including indoor factors in assessments of a chemical’s persistence in an entire region, for both LCMs and a wider range of lowly-volatile, indoors-released chemicals.

### 3.17.P-Tu303 Microbial Synergy: Exploring Bioremediation and Microplastic Dynamics in Environmental Resilience

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Solutions to address plastic pollution may exist at the microscopic scale. We employ an interdisciplinary approach to understand how microorganisms utilize organic materials as sources for energy. Many organic compounds are susceptible to partial or complete carbon chain breakdown through enzyme activity by naturally occurring microorganisms [1,3]. While multiple factors influence the extent and speed of this degradation, evidence suggests bioremediation, a method employing biological processes to remove pollutants and detoxify contaminated environments, may be a promising approach to address plastic and microplastic (MP) polluted environments [2]. Strains of bacteria capable of degrading Low Density Polyethylene (LDPE) may also have the potential to degrade mineral oil (MO) due to their shared linear hydrocarbon nature and the potential bioavailability of oil. This evidence emerged from the bacterial cultures in Petri dishes carried out at regular intervals to constantly monitor growth or any variations. Of the 40 strains of bacteria we identified as capable of degrading LDPE, we focused our study on the four most promising. We recorded the regular degradative activity of the chosen bacterial strains through specific ATP tests. Our study’s findings provide valuable insight for selecting bacterial species with high degradation capacity. However, further research is necessary to better comprehend their mechanisms and potential for environmental remediation.

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### 3.17.P-Tu304 Effect of Internal Hydrophilic Groups of Surfactants on Biodegradability and Ecotoxicity: An Example of a Newly Developed Surfactant, Bio IOS

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Recently, a new sustainable anionic surfactant called Bio-based Internal Olefin Sulfonate (Bio IOS) has been developed. It has a unique structure of long hydrophobic alkyl chains (C16 to C18) with two types of hydrophilic groups in its midsection, which distinguish it from other conventional anionic surfactants. However, the effects of the specific structural features of the surfactants, especially the internal hydrophilic groups on its environmental properties and the consequent effects on the environment remain unclear. In this study, we investigated the environmental fate and ecotoxicity of Bio IOS, and elucidated whether different types and positions of hydrophilic groups will affect biodegradability and ecotoxicity. Biodegradation studies demonstrated that Bio IOS is readily biodegradable with >99.5% removal in wastewater treatment activated sludge and a fast

half-life of 5.8 h in river water; the excellent biodegradability is likely due to the high water solubility attributed to the internal hydrophilic groups. Meanwhile, moderate ecotoxicity was observed, whereby the 50% lethal and effect concentrations of the three freshwater species were above 1 mg/L. Ecotoxicity studies with different types and positions of hydrophilic groups revealed that hydroxyalkane sulfonate was less toxic and that toxicity was reduced in the presence of more internally located hydrophilic groups. These findings suggest that the hydroxyl group and the internal position of hydrophilic groups that constitute the molecular configuration resembling two separate shorter alkyl chains may reduce the adverse effects on organisms despite the long alkyl chains. Our study suggests that it is the internal hydrophilic group that may impart environmental properties. In this presentation, the importance and positive aspects of the internal hydrophilic group in surfactants on biodegradability and ecotoxicity will be discussed.

### **3.17.P-Tu305 Biodegradation and biotransformation products of oxygen-containing Liquid Organic Hydrogen Carriers (oxo-LOHCs)**

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Liquid Organic Hydrogen Carriers (LOHCs) are very promising vectors for hydrogen storage and transportation as they have high gravimetric hydrogen storage capacity. The LOHC systems consist of a pair of H<sub>2</sub>-lean, typically (poly)cyclic aromatic compounds, and H<sub>2</sub>-rich form, (poly)cyclic aliphatic compounds. Pilot projects of energy storage and transport operate at the scale of hundreds of tons of LOHC per year and could increase considerably in the future. The search for LOHC systems with excellent technological performance and acceptable hazard profiles is of paramount interest. Recently novel oxo-LOHC systems that have demonstrated favorable technological properties yet their environmental impacts are unknown. This study presents the ultimate biodegradation, primary biodegradation, and biotransformation products of a series of oxo-LOHCs. Our results show that monocyclic oxo-LOHCs are readily biodegradable (mineralization > 60%) while bicyclic oxo-LOHCs are not. Some of the bicyclic oxo-LOHCs are inherently biodegradable while others show minimal mineralization, especially H<sub>2</sub>-rich forms (<20%). In the benzophenone-based LOHC system, four compounds showed 100% primary biodegradation, whereas the other two oxo-LOHCs lacking a phenyl ring showed lower degradation. Methylbenzophenones showed a relatively lower biodegradation rate, suggesting that methyl substitution decreases the biodegradability with some compounds showing neither ultimate nor primary degradability. Additionally, high-resolution mass spectrometry identified three types of hydroxylated benzophenones following benzophenone biodegradation that could exhibit endocrine activity. The primary degradation half-life of most oxo-LOHCs is shorter than 35 days. These findings offer valuable guidance for LOHC developers in selecting or designing the least harmful substances with outstanding performance as hydrogen carriers.

### **3.17.P-Tu306 Degradation of Metribuzin in a Tropical Soil Amended with Sugarcane Straw Biochar**

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Biochar-amended soils influence the degradation of herbicides depending on the pyrolysis temperature, application rate, and feedstock used. Metribuzin is a pre- and post-emergent herbicide, a selective residual of the triazinone group, with the ability to effectively control a wide spectrum of eudicot weeds. The objective of this study was to evaluate the influence of sugarcane straw biochar (BC) produced at different pyrolysis temperatures (350 °C, 550 °C, and 750 °C) and application rates in soil (0, 0.1, 0.5, 1, 1.5, 5, and 10% w/w) on metribuzin degradation. Detection analysis of metribuzin in the soil to find time for 50% and 90% metribuzin degradation (DT<sub>50</sub> and DT<sub>90</sub>) was performed using high-performance liquid chromatography (HPLC). BC350 °C-amended soil at 10% increased the DT<sub>50</sub> of metribuzin from 7.35 days to 17.32 days compared to the unamended soil. Lower application rates (0.1% to 1.5%) of BC550 °C and BC750 °C decreased the DT<sub>50</sub> of metribuzin to ~4.05 and ~5.41 days, respectively. The addition of low application rates (0.1% to 1.5%) of sugarcane straw biochar produced at high temperatures (BC550 °C and BC750 °C) resulted in increased metribuzin degradation and may influence the residual effect of the herbicide and weed control efficiency.

Keywords: application rate; carbonaceous material; degradation time; herbicide residual; pyrolysis temperature.

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### **3.17.P-Tu307 Degradation of glyphosate in drinking water with 60Co gamma radiation using LC-MS/MS**

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Glyphosate (N-(phosphonomethyl)glycine) is a systemic, post-emergence, non-selective herbicide. Due to the control of a wide range of weeds and transgenic events (crops resistant to this herbicide), glyphosate has become the most important and widely used herbicide in the world. However, due to its toxicity, especially to aquatic beings, it is necessary to study the control and degradation of glyphosate in water bodies, since it is more persistent than in soil. The aim of this study was to evaluate the degradation of glyphosate in drinking water using gamma radiation. This study was carried out using a sample of tap water from Viçosa, MG, Brazil. Initially, it was verified that the sample did not contain the herbicide, and then a dose of glyphosate of 0.5 mg L<sup>-1</sup> was added. Subsequently, the samples were subjected to different doses of gamma radiation (0, 0.5, 1.0, 2.5, 5.0,

and 10 kGy) in an irradiator of the type Gammacell 220 Excel, MDS, Nordion (Atomic Energy of Canada Limited - AECL, Ottawa, Ontario, Canada). The procedure was performed at room temperature with a dose rate of 0.08 kGy/h (source power: 215.95 Ci). This equipment has a high capacity to emit gamma rays through a Cobalt 60 ( $^{60}\text{Co}$ ) source, located in Piracicaba, SP, Brazil. Glyphosate was quantified using liquid chromatography coupled with mass spectrometry (LC-MS/MS). The dose constant ( $k$ ) was determined from the slope of  $\ln(C_D/C_0)$  versus absorbed dose ( $D$ ) by considering the pseudo-first order degradation kinetics;  $-\ln(C_D/C_0) = kD$ . The value of  $k$  was used to find out the amount of irradiation doses required for 50 ( $D_{0.5}$ ) and 90% ( $D_{0.9}$ ) degradation of each compound by using equations:  $D_{0.5} = \ln(2)/k$  and  $D_{0.9} = \ln(10)/k$ , respectively. The pseudo-first order model of the glyphosate degradation kinetics study was very well adjusted. The  $D_{0.5}$  was 8.97 kGy and the  $D_{0.9}$  of glyphosate was 29.82 kGy. The highest dose of irradiation applied (10 kGy) was able to degrade ~63% of glyphosate, but the presence of 20% of its metabolite aminomethylphosphonic acid (AMPA) was detected. Thus, this study concluded that the use of gamma radiation is a possibility for the degradation of glyphosate in water from treatment plants, but it is necessary to use higher doses.

Keywords: decontamination, organic pollutant, metabolite, herbicide.

Funding: This research was funded CNPq.

### 3.17.P-Tu308 Oxygen-containing Liquid Organic Hydrogen Carriers (oxo-LOHCs) as materials for storage of green hydrogen – environmentally acceptable, circular, and free of critical raw materials?

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Liquid Organic Hydrogen Carriers (LOHCs) are very promising vectors for hydrogen storage and transportation as they have high gravimetric hydrogen storage capacity. The LOHC systems consist of a pair of  $\text{H}_2$ -lean, typically (poly)cyclic aromatic compounds, and  $\text{H}_2$ -rich form, (poly)cyclic aliphatic compounds. Pilot projects of energy storage and transport operate at the scale of hundreds of tons of LOHC per year and could increase considerably in the future. The search for LOHC systems with excellent technological performance and acceptable hazard profiles is of paramount interest. Recently novel oxo-LOHC systems that have demonstrated favorable technological properties yet their environmental impacts are unknown. This study presents the biodegradation, ecotoxicity, mobility, and the *in vitro* bioconcentration potential of a series of oxo-LOHCs. Our results show that monocyclic oxo-LOHCs are readily biodegradable (mineralization > 60%) while bicyclic oxo-LOHCs are mostly inherently biodegradable. In the benzophenone-based LOHC system, four compounds showed 100% primary biodegradation, whereas the other two oxo-LOHCs lacking a phenyl ring showed lower degradation. Methylbenzophenones showed a relatively lower biodegradation rate, suggesting that methyl substitution decreases the biodegradability with some compounds showing neither ultimate nor primary degradability. Additionally, high-resolution mass spectrometry identified three types of hydroxylated benzophenones following benzophenone biodegradation that could exhibit endocrine activity. Ecotoxicity tests conducted with *Daphnia magna* (water flea) and *Raphidocelis subcapitata* (green algae) showed that the toxicity of oxo-LOHCs is generally moderate belonging to the acute 2 and acute 3 categories according to the Globally Harmonized System classification. Notably, some methylbenzophenones exhibited high toxicity to green algae (Acute 1:  $\text{EC}_{50} < 1 \text{ mg/L}$ ). oxo-LOHCs are expected to be generally mobile in the soil environment based on their log  $K_{oc}$  values (mainly below 3.0). Lastly, oxo-LOHCs show a low bioconcentration potential due to their low measured membrane-water partition coefficients (log  $K_{mw}$ ). These findings offer valuable guidance for LOHC developers in selecting or designing the least harmful substances with outstanding performance as hydrogen carriers.

### 3.18.A Next-Generation Urban Water Management: Improved Understanding of the Fate of Micropollutants, Transformation Products, Pathogens, and Antimicrobial Resistance

#### 3.18.A.T-01 Occurrence of pollutants of emerging concern in urban runoff samples

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Emerging pollutants are contaminants of anthropogenic origin that have been studied with great interest over the last 20 years, mainly in wastewater. Among them, persistent and mobile compounds (PMs) are contaminants of new emerging concern due to their lack of information. As happened in the last century with persistent, bioaccumulative and toxic compounds (PBTs), PM compounds pose a great threat to the water cycle. One of the main sources of this pollution are the effluents of wastewater treatment plants, but there is more like runoff after rain events in cities. Runoff water in cities is considered one of the main pollution vectors, causing considerable deterioration of the receiving water masses. However, the analysis of pollutants with such a wide range of polarities is very challenging. Therefore, in this work, we have created a methodology able to preconcentrate and measure, detecting 75 compounds in urban runoff samples. The total contamination of the samples ranges from 5 - 76  $\mu\text{g/L}$ , so contamination in runoff water in cities must be taken into account. Among analysed pollutants, 9 analytes were detected with average concentrations above 1000  $\text{ng}\cdot\text{L}^{-1}$ : mono-methyl phthalate, 2,3-dimethylbenzenesulfonic acid, naphthalene-1-sulfonic acid, *e*-caprolactam, tris(2-butoxyethyl) phosphate, glufosinate, docusate, 1,3-diphenylguanidine and

tolyltriazole; all of them used as industrial compounds or in the manufacture of objects except the herbicide glufosinate and the laxative docusate. Overall, this research aims to contribute to a better understanding of the pollutants present in stormwater runoff and provide insights into effective strategies for their identification and mitigation.

### **3.18.A.T-02 Lysimeter Experiments to Investigate the Retention and Degradation of the Urban Biocides Terbutryn, Diuron and Octylisothiazolinone in Typical Urban Surface-Soil Interfaces**

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Urban biocides, such as terbutryn, diuron, and octylisothiazolinone, are emitted from facade paints and plasters, reaching groundwater through infiltration through permeable surfaces. While the presence of urban biocides in groundwater is established, their pathways and reactive behavior across surface-soil interfaces remain poorly understood. This study employs field lysimeter experiments and reactive transport modelling to examine urban biocide reactive transport in soil. The results indicate that pavement and drainage gravel had insufficient capacity to retain biocide fluxes, releasing 10-50% of the initial mass to deeper soil layers. In contrast, mass transfer through vegetated soil systems was limited, with less than 0.5% released. After 207 days, up to 9% of the initially applied terbutryn mass was extractable in all lysimeters, with a substantial amount (7-18%) still available for further leaching to groundwater. Compound-specific isotope analysis enabled to evaluate the degradation contribution to total dissipation, revealing a higher degradation extent in the pavement than in the gravel lysimeter. Given the time limitations of outdoor experiments, reactive transport models helped predicting the primary entry paths and mass fluxes of urban biocides through urban surfaces to evaluate associated risks over years. Thus, continuous biocide release after rain events from a 10 m<sup>2</sup> facade, was employed to assess the reactive transport through various surface-soil interfaces to groundwater over an 8-year period. Terbutryn, along with transformation products, reached deeper soil layers (>30 cm) through pavement and gravel, accounting for 22% and 36%, respectively, over the 8-year period. Only 0.2% of terbutryn leached through vegetated soils. In summary, this study represents a fundamental step towards integrating biocide leaching into the design of sustainable stormwater management approaches to evaluate risks of groundwater contamination.

### **3.18.A.T-03 Sources of Persistent and Mobile Chemicals in Municipal Wastewater: A Sewer Perspective**

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By definition, persistent and mobile (PM) chemicals are poorly degraded by microbial processes and hardly sorb to natural surfaces. This is why PM chemicals can spread in the water cycle and may be found throughout the aquatic environment. Monitoring studies demonstrated the occurrence of PM chemicals in various environmental compartments. All these studies indicate the widespread presence of some PM chemicals like acesulfame or 1-cyanoguanidine which appear in almost every sample while others were detected more sporadically but then partially in high concentrations. Since many of these chemicals are only partially removed by wastewater treatment knowledge of their sources becomes essential to devise appropriate mitigation options like the implementation of an on-site pretreatment of the wastewater or a more focused search for replacement chemicals for specific applications if their release is found to be problematic. Furthermore, a better understanding of sources may inform future monitoring and screening campaigns by aiding in a tailored sample selection.

In this study 67 PM chemicals were investigated to explore the potential of sewer sampling for identifying sources in municipal wastewater. In total, 37 analytes (54%) were detected in at least one of the wastewater sample. The sewer samples analyzed were highly variable in both, composition and concentration: 22 of the 37 detected compounds analyzed (49%) were detected in less than 50% of the samples, while 15 compounds (41%) spanned up to three orders of magnitude in concentration. For source identification the samples were categorized in clinical, domestic, and industrial wastewater. The generated data showed that PM chemicals analyzed (a) originated from diffuse (e.g. vincubine) or point sources (e.g. 1-cyanoguanidine) and (b) what kind of point source a chemical originated from. While many chemicals categorized as industrial were also diffusely emitted by households, high concentration emissions could be linked to specific applications and industries for some PM chemicals like 1-cyanoguanidine.

### **3.18.A.T-04 Modeling the fate of CEC during wastewater treatment processes by using fluorescence**

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In this study fluorescence measurements suitable for real-time monitoring of CEC removal from wastewater during conventional and advanced oxidation processes (AOPs) has been investigated. The study focused on tracking and modelling the fate of CEC during the treatment processes by using fluorescence measurements and Artificial neural network (ANN).

Several samples were collected at two different municipal WWTPs and at a AOP pilot plant operated downstream of a water reuse facility (WRF) for agricultural purposes. In particular, some samples were collected before and at different stages of AOP pilot plant operated at different flowrates and treatment train.

The organic micropollutants were detected by means of an Orbitrap mass spectrometer coupled with high resolution liquid chromatography with a non-target suspect-screening methodology. Fluorescence data were collected using a Shimadzu RF-5301PC fluorescence spectrophotometer (Kyoto, Japan) with the scanning range from excitation wavelength 220 nm–450 nm and emission wavelength from 250 nm to 580 nm. Five fluorescence peaks were selected and used for CEC modeling. These are referred as I1 (ex 225, em 290), I2 (ex 230, em 355), I3 (ex 245, em 440), I4 (ex 275, em 345), I5 (ex 345, em 440).

ANN model has been developed using Matlab software with ANN toolbox to match the measured and the predicted concentrations of CEC. The input parameters selected for the model included the abovementioned five fluorescence peaks of EEM. A feed-forward three-layer trained with Levenberg-Marquardt with a log-sigmoid transfer function for the hidden layer and a pure-linear transfer function for the output layer has been used. The network has been fed with three subsets of data, with 50% being used for training, 25% for testing the performance and 25% for validation to avoid the overfitting.

Obtained results show that selected fluorescence indexes can be used to monitor and model the fate of CEC during wastewater treatment processes. For instance, a strong linear correlation between the sum of CEC (normalized concentration) and a selected fluorescence index (normalized values) obtained at varying treatment process (AOPs based) and operational day.

The CEC concentrations were also well predicted by the ANN-based model that incorporated as input parameters the values of I3, I4, and I5. Indeed, the ANN-based model can predict the CEC concentrations with a high determination coefficient ( $R^2 > 0.9$ ).

### **3.18.A.T-05 Phase Separation of Anaerobic Digestion to Elucidate the Transformation Products of Organic Micropollutants during Acidogenesis and Methanogenesis**

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The presence of organic micropollutants (OMPs) in wastewater treatment plants (WWTPs) has been frequently reported and the risks inherent to their environmental exposure by animals and humans is being increasingly elucidated. These OMPs can be completely mineralized or undergo various processes of cometabolic transformation mediated by different microbial communities in WWTPs. This study aimed to elucidate the biotransformation products (BTPs) from OMPs during anaerobic digestion, emphasizing the specific contribution of acidogenesis and methanogenesis in the transformation products of each compound. The experimental apparatus was composed of two anaerobic fixed-film bioreactors: a single-phase (SP) methanogenic and a two-phase (TP) sequential acidogenic-methanogenic reactor. The bioreactors were fed with wastewater spiked with  $10 \mu\text{g L}^{-1}$  of ten selected OMPs: carbamazepine, naproxen (NPX), diclofenac (DCF), ibuprofen, acetaminophen, metoprolol, sulfamethoxazole, ciprofloxacin, methylparaben and propylparaben. Influent and effluent liquid samples from each bioreactor were processed by solid phase extraction and analyzed by LC-HRMS (Q-Exactive QOrbitrap). By applying a suspect screening analysis, for the potential BTPs the experimental MS2 spectra obtained were compared with fragmentation patterns found in databases (e.g., Massbank) to confirm the chemical structure of each compound. In summary, fourteen BTPs were confidently identified and it was possible to establish some possible transformation pathways, emphasizing the main chemical reactions involved, e.g., demethylation of NPX and hydroxylation of DCF to generate demethyl-NPX and hydroxy-DCF, respectively. Apart from the transformation pathways, we compared the BTPs found in the SP and TP reactor. We observed that certain BTPs are generated during the acidogenic phase and further utilized during methanogenesis (e.g., demethyl-NPX). On the contrary, another subset of BTPs is generated under acidogenesis but exhibits an increase in concentration in the TP-reactor effluent (e.g., hydroxy-DCF). As a conclusion, it was found that the OMPs transformation was closely linked to the fermentative cometabolism of organic acid production, since all BTPs were found in acidogenic condition. The TP anaerobic bioreactor enhanced the biodegradation of OMPs in wastewater, but greater attention should be given to the potential risks of the generated BTPs in the effluent stream from these reactors to the environment.

### **3.18.B Next-Generation Urban Water Management: Improved Understanding of the Fate of Micropollutants, Transformation Products, Pathogens, and Antimicrobial Resistance**

#### **3.18.B.T-01 Development of an automated workflow for conducting degradation experiments and elucidating transformation products**

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Organic micropollutants (OMPs), including pharmaceuticals and their transformation products (TPs), are prevalent in aquatic environments, and challenge conventional water treatment processes. Advanced oxidation processes such as UV photolysis serve as an effective strategy to remove OMPs by direct and indirect photolysis mechanisms. However, the non-selectivity of OH radicals raises concerns about the unpredictable formation of various TPs with unknown toxicity, requiring a comprehensive understanding of degradation pathways. Liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) paired with non-target analysis (NTA) is typically used for comprehensive screening of OMPs and their TPs. While powerful, NTA faces challenges such as complex data interpretation, low concentrations of compounds of interest, lack of reference standards, and the need for sophisticated data analysis workflows. This study aimed to establish comprehensive workflows for degradation experiments and TP elucidation, combining an automated photodegradation setup ('TooCOLD') with software NTA workflows ('patRoom 2.0'). The TooCOLD setup integrates UV light and LC-HRMS, eliminating manual labor and expediting experimental processes. The TP screening was based on patRoom, an open source software platform to perform comprehensive NTA. These workflows were then applied to a case study on the photodegradation of the pharmaceuticals flecainide, metoprolol, sulfamethoxazole and phenazone. The study examined UV photodegradation in the presence and absence of H<sub>2</sub>O<sub>2</sub> and natural organic matter (NOM), assessing parent chemical dissipation and employing NTA to identify TPs. The integration of these methodologies provided insights into transformation processes and TP formation during water treatment, under controlled conditions. The studied pharmaceuticals exhibited diverse degradation patterns under the investigated treatment conditions. Preliminary NTA results revealed a total of 256,784 features, which were reduced to 38 features through various prioritization steps, and tentatively identified using various suspect and unknown screening approaches. This work demonstrated how comprehensive workflows to systematically perform degradation experiments with complementary screening approaches can be used to effectively elucidate a significant number of structurally diverse TPs for different parent compounds.

### **3.18.B.T-02 Assessing the Fate and Behaviour of Micropollutants During Advanced Wastewater Treatment for Potential Water Reuse Applications**

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The ever-increasing freshwater stress across the globe requires innovative solutions to ensure a high-quality and constant water supply for the public, industry, agriculture, and nature. Reusing treated wastewater offers in this instance great potential to support existing water supply systems during periods of increased freshwater pressure either due to an increase in demand or decrease in water availability.

The focus of wastewater reuse related health concerns has so far been on potential adverse effects caused by microorganisms. Contrary, the numerous chemical contaminants that are present in wastewater treatment plants effluent, remain poorly understood during water reuse. Therefore, to address and limit potential risks caused by these chemical pollutants, their fate and behaviour during reuse needs to be comprehensively assessed.

To study the potential reuse of treated municipal wastewater effluent after advanced treatment, a pilot plant was installed on the wastewater treatment plant (WWTP) in Wervershoof, the Netherlands. The pilot plant receives WWTP effluent as intake water and treats it by a combination of ozonation, ceramic membrane filtration, and activated carbon. Over the summer of 2022, 90 samples were taken at this pilot plant before and after each individual treatment step. Samples were enriched by solid-phase extraction and were subjected to several *in vitro* bioassays covering multiple toxic endpoints. Furthermore, chemical target analysis of 98 micropollutants by liquid chromatography trapped ion mobility spectrometry coupled to a time of flight mass spectrometer is currently still ongoing. This analysis will help to assess removal efficiency of target compounds and will be supplemented by suspect as well as non-target screening.

This talk will emphasize the advantage of coupling chemical analysis and effect-based analysis for the analysis of complex mixtures. Their integration allows to address known pollutants to ensure compliance with existing regulations, while simultaneously accounting for unknown chemicals and the entire mixture. Therefore, extending the covered chemical regulatory space, which ultimately improves the overall risk assessment. The findings will especially be interesting for water authorities that aim to initiate water reuse applications as appropriate treatments options along with monitoring options are presented that will help to understand and minimise potential risks.

### **3.18.B.T-03 Qualitative and quantitative spatiotemporal analysis of new psychoactive substances in Slovenia through the analysis of influent wastewater**

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New psychoactive substances (NPS) are a constantly evolving class of psychoactive substances which were originally sold as legal alternatives to conventional illicit drugs such as ecstasy and cannabis while bypassing legal restrictions and are thus of international public health concern. The continuous appearance of new NPS derivatives on the market is making the

monitoring and surveillance of NPS challenging using conventional socio-epidemiological methods. A complementary approach that could deliver additional information on the spatiotemporal consumption patterns of NPS is wastewater-based epidemiology (WBE). WBE measures trace concentrations of biomarkers in influent wastewater and converts these to per capita mass load estimates (mg/day/1000 inhabitants) of drugs using pharmacokinetics, daily flow rates of wastewater and population in the catchment area. In this study, influent wastewater samples from seven wastewater treatment plants in Slovenia (Ljubljana, Maribor, Velenje, Domžale-Kamnik, Koper and Novo Mesto) were analysed during six different sampling periods: summer 2019, winter/spring 2020, winter/spring 2021 and spring 2022. Following solid-phase extraction, two liquid chromatography-mass spectrometry (LC-MS) methods were utilised: targeted LC-MS/MS method of more than 70 NPS as well as an LC-quadrupole time-of-flight (LC-QTOF) suspect screening method using a HighResNPS database of more than 2000 NPS and illicit drugs. Our analysis allowed a spatiotemporal analysis to determine the extent of NPS use in Slovenia. Five NPS were semi-quantified across all sites: 3-methylmethcathinone (3-MMC), 4-fluoroamphetamine (4-FA), eutylone, mephedrone, and mitragynine. The highest number of residues (n=5) was measured in Koper, followed by Domžale-Kamnik and Ljubljana. Following the suspect screenign method, at least 10 additional compounds were detected. This wastewater-based study provides an insight into the NPS market in Slovenia, including before, during and after the COVID-19 pandemic, while combining qualitative and quantitative methods gives a more comprehensive overview of their use.

### **3.18.B.T-04 Wastewater Surveillance for Real-Time Micropollutant Monitoring and Advanced Treatment Assessment**

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Recent adoption of advanced treatment sees the use of efficient techniques for CEC elimination at WWTP outlets, such as granulated activated carbon (GAC) filters and ozonation. Most evaluation of such treatment relies on grab sampling measurements for only a select group of chemicals. Given the temporal variability associated with CEC discharge, treatment now requires real-time monitoring strategies to characterise micropollutant fate at times of micropollutant surge. ToxMate online biomonitoring, using multispecies (Erpobdella, Gammarus, Radix) organism avoidance behaviour as a biomarker, offers effluent characterisation using an effect-based non-targeted approach. The objective of the communication is to present results and variability in advanced treatment performance over a continuous 18-month surveillance period, as well as current laboratory work on fingerprinting for the identification of CECs.

ToxMate biomonitoring was used at an advanced treatment site (120,000-population equivalent WWTP) where biological treatment was followed by ozone treatment and then GAC tanks, one ToxMate after each respective treatment for comparative monitoring (3 total). Parallel sampling of model micropollutants showed moderate elimination after ozonation and good overall elimination (post GAC). However, there was both temporal and molecule dependent variability in the elimination. Our results from the long term monitoring highlight this variability, where residence times are used to estimate correlation in micropollutant surge, in some cases showing persistence of the outgoing pollution or even the appearance of avoidance reactions, perhaps related to treatment byproducts (ie.metabolites).

Lab studies for repeated spikes tested for over 40 micropollutants were modelled with a functional approach, grouping responses into clusters known as behavioural fingerprints. Our current research looks to reveal structural similarities within fingerprints, that could improve identification of CEC nature at sites such as the described WWTP. Coupling this method and the rare insight into the characterisation of pollutant fate in advanced treatment provides an opportunity for real-time alerting in WWTPs in the case of more persistent micropollutant surge, perhaps indicating temporary periods where applications such as re-use should be avoided. It also suggests the need for a review of current chemical analysis used to evaluate overall micropollutant elimination in advanced treatment.

### **3.18.B.T-05 Is the conventional treatment process sufficient to decrease the ecotoxicological effect of wastewater?**

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Aquatic bodies face increasing levels of pollution worldwide, particularly from specific contaminants called micro-pollutants that can generate adverse effects for ecosystems and human health. Considering this fact, it is well known that just 40% of surface water bodies in Europe meet the requirements for good ecological and chemical status and in Germany the situation is even worse with just 10% of surface waters. Many studies confirm that one of the main entry path of these micro-pollutants into the aquatic environment is through treated and untreated wastewater.

It is therefore important to analyze the effectiveness of the WWTPs in eliminating micropollutants and other chemical stressors to mitigate the negative impacts of the treated wastewater (WW) in the aquatic ecosystems. For that reason, in the present study, we assessed, through effect based methods, two conventional wastewater treatment plants located in the Hessian Ried in Germany. The tests performed in this study were baseline toxicity with *Aliivibrio fischeri*, mutagenicity assay with *Salmonella typhimurium* and endocrine-disruption activity with *Saccharomyces cerevisiae*. These activities were measured at four different points in each WWTP: upstream, after mechanical treatment, after biological treatment, and downstream during spring and fall. In general, the results showed that the untreated WW had a very high baseline toxicity and also high endocrine



and mutagenic activities; however the conventional wastewater treatment (consisting of mechanical and biological treatment with nitrification, denitrification and phosphate precipitation) reduces in more than 90% the baseline activity and in 80% the endocrine activities. Despite these high elimination rates, the remaining baseline toxicity, the endocrine, dioxin-like and mutagenic activities of the conventionally treated WW was so high that negative effects on the two receiving waters were to be expected. These results imply that a further advanced wastewater treatment is urgently needed to guarantee a safe effluent in both streams because the conventional treatment process is not sufficient to decrease the ecotoxicological effect of the wastewater.

### **3.18.C Next-Generation Urban Water Management: Improved Understanding of the Fate of Micropollutants, Transformation Products, Pathogens, and Antimicrobial Resistance**

#### **3.18.C.T-01 Evaluating membrane bioreactor treatment for the elimination of emerging contaminants using different analytical methods**

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Wastewater treatment plants (WWTPs), originally not designed for the elimination of contaminants of emerging concern (CECs), are grappling with alternative strategies such as membrane bioreactor (MBR) technology to efficiently eliminate CECs and mitigate their potential environmental impact. This study evaluated the efficiency of two secondary treatments, conventional activated sludge (CAS) and MBR treatments, implemented at the largest WWTP in the Basque Country (Galindo, Biscay). Employing a suspect screening approach through liquid chromatography tandem high-resolution mass spectrometry (LC-HRMS) and multitarget analysis via both, LC-HRMS and gas chromatography-mass spectrometry (GC-MS), nearly 200 compounds were detected in both WWTP effluents. Removal efficiency (RE) evaluation revealed that merely 16 micropollutants exhibited enhanced removal by MBR (RE > 70 % or 40 – 60 %). The environmental risk, based on model calculation, posed by non-eliminated compounds post-treatment remained similar in both cases. Notably, anthracene, clarithromycin, bis(2-ethylhexyl) phthalate (DEHP), and dilantin were pointed out as the most potentially toxic compounds, exceeding the threshold of RQ > 1. The Microtox® bioassay experimentally confirmed the efficiency of MBR in baseline toxicity removal, while suggesting a similar performance of CAS treatment. These minimal treatment differences prompt reconsideration of the worthiness of MBR treatment, highlighting the essential quest and development of more efficient alternative treatment methods.

#### **3.18.C.T-02 Bioaccumulation Of Emerging Contaminants: A Fishy Story**

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The ubiquitous occurrence of contaminants of emerging concern, as well as their environmental and health impacts, is an issue of high concern. Therefore, aquatic organisms living in water bodies affected by the effluent of wastewater treatment plants (WWTPs) are exposed throughout their lives to mixtures of various compounds. Effluent-dominated ecosystems represent worst-case scenarios for the exposure and potential adverse effects. The uptake of contaminants from polluted water or via food can lead to bioconcentration/bioaccumulation of these compounds in aquatic organisms.

Measurement of pharmaceuticals in fish has been proposed as a way of assessing potential hazards by comparing observed levels in fish blood plasma to human therapeutic plasma doses. The apparent volume of distribution ( $V_D$ ) is a theoretical value representing how extensively a drug distributes throughout the body.  $V_D$  values can be used to estimate body burden by analyzing contaminants in fish plasma. In the current study, we employed an ecosystem-based treatment system, specifically employing a treated wastewater pond, which receives 100% treated WWTP effluent as a tertiary treatment component. The objective of this study was to calculate  $V_D$  for a broad range of contaminants for two fish species and estimate the time needed for their elimination from the fish body, aiming to provide recommendations for the safe consumption of fish produced in wastewater.

We detected a few of the studied contaminants in fish from wastewater pond. Only trace levels were observed for most of them, which were comparable with those found in wild fish. Moreover, some compounds were eliminated after a few days spent in the clean water. The results indicate that reusing wastewater for fish production is not only sustainable but also a relatively safe option with respect to the contaminants of emerging concern. Calculated  $V_D$  values will allow us to estimate the body burden using the analysis of fish blood plasma only, avoiding killing the fish.

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### **3.18.C.T-03 On the Trail of Persistent Mobile and Toxic Compounds in Urban Stormwaters: Case Study of Barcelona Municipality**

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Persistent, mobile, and toxic (PMT) chemicals are contaminants of recent interest for urban water quality, increasingly at risk due to the rise in urbanization and extreme events. Despite many polar and persistent compounds have already been detected in various water sources, scarce monitoring data has hindered their comprehensive resolution. Conventional water treatments frequently prove ineffective in their removal, given the persistence and their high mobility, they can end up in both ground and surface waters. In 2019, German environmental authority UBA issued a list of PMT substances registered under the REACH, offering a relative prioritization grounded in a preliminary emission assessment.

Stormwater harvesting represents a promising resource, particularly in densely populated arid regions marked by water scarcity. Simultaneously, it is acknowledged as a significant driver of contamination. This holds true case for “sponge cities”, where urban planning and design strategies are crucial for the sustainable management and utilization of rainwater. Among these, installation of stormwater blue-green infrastructures (BGI) is a promising practice in order to mitigate contamination, while recharging receiving urban aquifer and containing combined sewer overflow (CSO).

Herein, urban stormwater “first flushes” from various sites over 3 districts across the municipality of Barcelona, were collected during the period March to April 2022. Sampling design included conventional and pedestrian streets runoff through an evaporative enrichment methodology employed for targeted screening using a LC-MS/MS method. 34 PMTs of urban interest were selected, 14 of them being reported also in the UBA list of 2019. In this preliminary analysis, we observed that all targets were detected in at least one sample, among them 5 chemicals as benzenesulfonamide, 1,3 diphenylalanine, di-n-butyl phosphate, tolyltriazole and TCPP, resulted to be the more abundant with median concentrations higher than 100 ng L<sup>-1</sup>. Moreover, BGI effluent resulted in a diminished contamination load.

Results will eventually undergo thorough analysis to evaluate the extent in spatiotemporal domain of contingent related contamination. PMTs substances impacting urban stormwaters must be monitored in order to prevent spread in surface and ground waters and enable their safe use for water supply.

### **3.18.C.T-04 The Journey of Organic Micropollutants from Water towards Soil and Crops in a Reclaimed Water Irrigation System**

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In the last decades, water scarcity has become a challenging issue, especially in arid and semi-arid regions, such as the Mediterranean basin. Agriculture is among the sectors threatened by water scarcity and reclaimed water for irrigation could be a promising alternative but organic micropollutants must be evaluated because they can make their way into the food chain and pose a challenge regarding food safety and human health. This work assessed the uptake of a variety of organic micropollutants, including pharmaceuticals, antibiotics, and endocrine disrupting compounds in three different matrices (water, soil, crops). Briefly, the wastewater treatment system consists of an upflow anaerobic sludge blanket reactor, a constructed-wetland system, and finally a tertiary treatment. The reclaimed water is collected in storage tanks and applied for irrigation on adjacent horticultural fields. First, the demonstration scale wastewater treatment plant in a water scarce area was sampled, in two different seasons and the removal efficiencies of the treatment steps were calculated. Afterwards, the organic micropollutant content was evaluated also in the soil and in three crops (lettuce, lavender, oregano) irrigated with the treated effluent of the wastewater treatment plant. The organic micropollutants were detected in the soils before and after the growth periods as well as in the corresponding control soils. As to the crops, reclaimed water irrigation resulted in slightly higher concentrations of the same contaminants in the leaves, whereas root composition was largely different. Finally, both environmental and human health risk assessments of the wastewater-derived organic contaminants detected in irrigation water were performed based on the obtained results. This study can provide an overall assessment as regards organic micropollutants from regenerated wastewater including in water scarce areas irrigation water, soil, and crops data.

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### **3.18.C.T-05 Assessing the Impact of Municipal Effluent Discharge on a Small Watercourse in Aartselaar, Belgium: A Multi-Level Evidence Approach.**

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Urban wastewater treatment plants (UWWTP's) in Flanders (Belgium) are compliant with the European Urban Wastewater Treatment Directive (UWWTD). However, recent evaluation of the directive identified residual pollution from urban sources as one of the three remaining challenges leading to a proposal for a revised UWWTD (91/271/EEC). Part of the remaining pollution is due to micropollutants, that impose risks for health and environment at low concentrations. The Flemish wastewater treatment utility (Aquafin) and the Flanders Environmental Agency (VMM) are monitoring micropollutants and ecotoxicity since May 2022 at the UWWTP of Aartselaar (BE) to gain insight in the contribution of UWWTP's to the aquatic ecotoxicity and the biotic communities present. The selected UWWTP treats domestic wastewater of about 60.000 population equivalents and discharges into the Grote Struisbeek, a small water body with a downstream flow rate of 0.2 to 1.3 m<sup>3</sup>/s. In dry periods, the discharge makes up 60% of the total flow in the water body, therefore contributing significantly to its water quality, its ecotoxicological status and its biotic communities. More than 150 chemical substances, as well as 10 toxic end points (both *in vivo* and *in vitro*) were monitored in effluent of the UWWTP and in both upstream and downstream surface water, following the recommendations concerning monitoring methodology of the Dutch government. Proper flow rate data is collected to calculate load contributions from the UWWTP through a mass balance. Due to inconsistency of analytical limits of quantification (LOQ) on the one hand and predicted no-effect concentrations (PNEC) on the other hand, analysis of chemical substances on its own was insufficient to make a proper judgement. Meanwhile, monitoring toxic end points and biological communities appears to provide useful insights concerning the impact of effluent discharge. For certain chemicals and effects, toxicity of municipal effluent is clearly affecting downstream water quality in the Grote Struisbeek, often exceeding an environmental trigger value, as is for example the case for estrogenic toxicity. Currently, a quaternary treatment step, including ozone and granular activated carbon, to remove micropollutants, is taken in operation at the UWWTP in Aartselaar. Continued monitoring will help to understand water quality improvements that can be achieved by a post-treatment in cases with a similar context as the UWWTP of Aartselaar.

### **3.18.P Next-Generation Urban Water Management: Improved Understanding of the Fate of Micropollutants, Transformation Products, Pathogens, and Antimicrobial Resistance**

#### **3.18.P-Mo280 Bioretention Cell Design for Trace Organic Contaminant Removal**

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Stormwater rapidly moves an "urban cocktail" of chemicals from the built to the aquatic environment, including many trace organic compounds (TrOCs). The risks posed by TrOCs to people and the environment depend on their toxicity and on their physicochemical properties, which influence how people and ecosystems are exposed. Bioretention cells, a green infrastructure technology, are traditionally designed primarily to provide hydrological benefits, but can also reduce loadings of some TrOCs to receiving bodies.

Herein, we used the Bioretention Blues model to simulate the fate and behavior of seven environmentally relevant TrOCs with representative log  $K_{OC}$  values between -1.5 to 6.74 in a bioretention cell for 28 design storms. Next, we tested combinations of eight design and management interventions and assessed the design potential and tradeoffs involved in optimizing bioretention systems for chemicals found in three illustrative use-cases. These use-cases represented a major highway next to a salmon-bearing stream receiving 6PPD-quinone ((N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone) and 6PPD; a residential area receiving TCEP (tris(2-chloroethyl) phosphate), PHE (phenanthrene), and B[a]P (benzo[a]pyrene); and an airport receiving PFOA (perfluorooctanoic acid) and (1H-) benzotriazole. We found that the optimal designs for the highway, residential, and airport scenarios reduced effluent loadings of all but the most polar compounds to <5% of influent mass, showing that substantial improvements in TrOC removal could be achieved through bioretention design. Further, our results suggest that the most important intervention for hydrophobic compounds was to increase the system area, while for hydrophilic compounds adding an amendment like biochar was by far the most effective. Our results show that the design of bioretention systems can allow them to effectively capture chemicals like 6PPD-quinone or PFOA (perfluorooctanoic acid), protecting important aquatic species like salmon and human health from chemical impacts.

#### **3.18.P-Mo281 Identification of priority contaminants in groundwater for their protection and pollution prevention**

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The increasing prevalence of global drought is a current challenge requiring urgent exploration of solutions to ensure water supply. In areas with limited restricted water availability and poor quality, groundwater bodies are an essential resource and require protection against chemical pollution. Indeed, Directive 2018/80/EU, which modifies Annex II of Directive 2006/118/EC, related with groundwater pollution and deterioration, emphasizes the need to define a comprehensive list of priority contaminants that may represent a potential risk to groundwater bodies. Thus, groundwater quality assessment grows

into an evident need and monitoring studies are needed to identify such substances for inclusion in surveillance lists. In this context, the Catalan Water Agency (ACA) has established a monitoring network to identify and quantify selected emerging pollutants at control sites, with the aim of assessing the risk they pose to groundwater bodies and evaluate their suitability to be included in a watch list for further monitoring.

A total of 231 sampling locations will be analysed, comprising 137 points in agricultural areas and 94 in sites with urban and/or industrial influence distributed throughout Catalonia during the 2022 – 2024 timeframe. In these samples, 11 indicator pharmaceuticals (PhACs) and 12 perfluoroalkyl substances (PFAS) in the urban and industrial sites, along with 12 veterinary antibiotics in the agricultural areas, were measured using Ultra High Performance Liquid Chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS).

Preliminary results have shown the detection of a wide range of PhACs, including primidone, sulfadiazine, sulfamethoxazole, and significant concentrations were observed, particularly for amidotrizoic acid, from 16 to 1374 ng/L and carbamazepine, from 1.30 to 168 ng/L. For PFAS, 7 analytes were identified, with perfluorooctanesulfonic acid (PFOS) notably standing out at a concentration of 1147 ng/L in a specific location. Finally, the presence of antibiotics is limited to a smaller number of sites, being sulfamethoxazole and sulfamethazine, the most ubiquitous compounds found at relatively low concentrations.

### **3.18.P-Mo282 How should we implement quaternary treatments in Wastewater Treatment Plants? A techno-economic analysis**

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Micropollutants are a class of compounds found at trace level in the environment. They are released by several sources, but wastewater treatment plants (WWTPs) represent one of the biggest, since they are not designed to remove micropollutants. Currently, the revision of the Urban Wastewater Treatment Directive (UWWTD) addresses the problem of micropollutants (MPs) released by WWTP: a specific set of pharmaceuticals and cosmetics are required to be removed with a minimum efficiency of 80%. Among the best available technologies suggested and largely studied there are adsorption and ozonation. These technologies are widely applied for drinking water production, but they are scarcely applied in WWTPs. Moreover, these processes can be implemented with different configurations, but there are no indications on the design and type of implementation in the revision of the UWWTD. Furthermore, in the future the directive could be modified to include more MPs in the set of the targeted MPs, such as PFAS.

With this study we propose an approach to evaluate the costs associated with quaternary treatments, depending on several factors. A model was developed in WEST (DHI A/S) to (i) predict the removal of MPs by ozonation and by adsorption on activated carbon, and (ii) evaluate the best configuration for the selected wastewater treatment plant, depending on the specific target removal to be approached. In detail, not only the 80% removal, as stated in the UWWTD, will be used as target, but also we will refer to the removal that allows to guarantee a negligible environmental risk in the effluent. For each configuration, CAPEX and OPEX will be estimated and the results will be compared in order to identify the best solution.

In particular, as for adsorption, two configurations will be considered based on the activated carbon size: Powdered Activated Carbon (PAC) with recirculation, and Granular Activated Carbon (GAC) in fixed-bed columns. Pore diffusion and surface diffusion are included in the model and competition with the organic matter is also considered, using the simplified Ideal Adsorbed Solution Theory (IAST) model. Different combinations of filtration, GAC/PAC, ozonation and clariflocculation are compared.

This study provides a useful approach to allow water utilities identifying best quaternary solutions for their own WWTP, based on influent characteristics, WWTP layout, target removal, and costs.

### **3.18.P-Mo283 Development of a holistic (bio)analytical platform to assess the hazard of transformation products formed during water treatment**

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Organic micropollutants (OMPs) are widely distributed throughout the aquatic environment and pose significant difficulties for (waste)water treatment. In (waste)water treatment facilities advanced oxidation processes (AOPs), such as UV photolysis, are implemented for the removal of OMPs with the use of ultraviolet (UV) light and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). When undergoing AOP treatment, contaminants tend to react and form unknown and potentially toxic transformation products (TPs). The main challenge in the characterization of unknown TPs consists of the development of a scalable and comprehensive method to detect and identify TPs, assess their potential (in-vitro) toxicity and link their formation to AOP and water characteristics. The development of such a method would allow us to obtain a better understanding of the conditions under which potentially hazardous TPs are being formed. Liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) combined with non-target screening (NTS) is typically employed for chemical analysis of complex environmental samples. While NTS is a powerful tool for environmental analysis, it faces challenges related to data complexity, potential for false results, limited

availability of reference standards, matrix effects and the need for significant computational resources. Addressing these issues is crucial for reliable identification and quantification of environmental contaminants. In this work, we present a (bio)analytical platform that relies on LC-HRMS, (in-silico) hazard assessment, high-throughput fractionation and effect-directed analysis (EDA), to detect and where possible characterize hazardous TPs formed during the (waste)water treatment. Our aim is to link the chemical and hazard space of TPs by understanding under which conditions they are formed and their potential (in-vitro) toxicity. For this purpose, we will implement in-silico approaches to predict the formation of TPs based on (predicted) molecular structures and enhance the discovery and identification rates of TPs. The first part of this study is focused on (i) defining the chemical space and hazard space in which we will operate the (bio)analytical platform, and (ii) testing available in-silico tools on our test data set to obtain a preliminary overview of expected TPs as well as their toxicity.

### **3.18.P-Mo284 AI Assistance for Chemical Analysis of PPCPs in Water and Wastewater: Highlights and Potentials**

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A critical step in suspect or non-targeted analysis is to tentatively ascertain the existence of compounds using HRMS data compared with spectral libraries and then confirm them by reference standard substances. The challenges arise when many compounds are deemed to be detected or when the reference materials are not readily available, especially in the case of transformation or degradation products in complex environmental or wastewater matrices. To address this and to enhance confidence, artificial intelligence (AI) methods have facilitated the prediction of the retention time (RT) and collision cross section (CCS). However, the models validated versus real water and wastewater samples have not been surveyed sufficiently.

This presentation reviews selected features of AI-assisted chemical analysis of pharmaceuticals and personal care products (PPCPs) applicable in water and wastewater real matrices and highlights the potential for further research into this domain. The results show the importance of external validation, preferably by environmental samples, since the best-selected AI method during training and internal blind testing over the dataset for model development can differ from the most suitable one for new compounds. We envisage that AI-assisted models for predicting CCS considered as a matrix- or case-independent factor, either as single output or combined with RT, will be applied for future studies more frequently, especially for unknown detection of PPCPs and their metabolites, as well as their transformation and biodegradation products in the effluent of wastewater treatment plants and surface waters.

### **3.18.P-Mo285 Monitoring of Contaminants of Emerging Concern in Alternative Sources for Indirect Potable Water Reuse in Barcelona**

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In the last decades, Catalonia and in particular the area of Barcelona has suffered several climate extreme events. Since the beginning of 2021, it has been immersed in a sustained situation of meteorological drought that has put significant pressure on its water resources. In an effort to find solutions to this situation, the Catalan Water Agency (ACA) has started to search for alternative water sources with the aim to increase the availability of pre-potable water in the Catalan River Basin District. As a first step in this process, water samples collected from several potential sources were subjected to a suspect screening study that allowed to identify and prioritise the presence according to risk of 57 contaminants of emerging concern (CECs) in them. As a follow up, the occurrence of these CECs in the potential water sources is currently being investigated in a more comprehensive way using target analytical methods. The results of the analysis of 16 of them, which include pesticides, pharmaceuticals, drugs of abuse, personal care products and industrial chemicals, is reported in the present work. Their analysis in waters from 3 investigated scenarios was performed by means of on-line solid phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS) using different methods. All compounds were detected in at least one sample with the exception of Atrazine-desethyl (values below LOD: 0.03 ng/L). The most frequently detected compounds, present in all samples, were Carbendazim, O-Desmethyl-venlafaxine, N,N'-Diphenylguanidine, 4+5-Methylbenzotriazole, and Galaxolidone. In terms of concentration, the most abundant compounds were Sitagliptin (concentrations up to 60 µg/L), Galaxolidone (up to 11 µg/L), 4+5-Methylbenzotriazole (up to 4.1 µg/L), and O-desmethyl venlafaxine (up to 4.1 µg/L). Contaminants loads decreased from wastewater to surface water and ground water. Principal Component Analysis of the data allowed to differentiate the samples according to location and type of water matrix. Preliminary assessment of the risk using human health-based thresholds suggest potential risk in the case of Galaxolidone, Sitagliptin, and Benzoilecgonina in some samples. The results of this study and those conducted in parallel for other contaminants will be used to ensure the functionality of the investigated scenarios as pre-potable water resources and to define the optimal treatment trains to implement in upcoming purification plants.

### **3.18.P-Mo286 Methods for Identifying Priority Chemicals in Wastewater and Estimating Their Relative Environmental Risks: A Critical Review**

**Sagar Thakali<sup>1</sup> and Jennifer K. Saxe<sup>2</sup>, (1)Kenvue, (2)KENVUE**

Researchers have proposed various approaches to identify and prioritise chemicals in wastewater and assess their relative

environmental risks, which are critical to understand for judicious attribution of sources and subsequent allocation of resources to mitigate exposure. This study will review the literature on existing prioritisation approaches for chemicals in wastewater and estimation of their relative environmental risks, with particular focus on their original study purpose, the underlying technical basis, and their strengths and limitations. Most existing approaches rely on a measure of risk for chemicals prioritisation, as typically represented by the ratio of the environmental concentration (exposure) to the ecotoxicity benchmark (effect). However, the identification/establishment of an appropriate ecotoxicity benchmark depends on number of factors, including quality and quantity of available data for a specific chemical and an accounting of associated uncertainty. The uncertainty in ecotoxicity benchmarks is well known to factor critically in the reliability of risk-based prioritisation of chemicals in wastewater. Hence, this review will identify key considerations when attempting to attribute chemical-related environmental risks, focusing particularly on the use of discrepant ecotoxicity benchmarks and associated implications on prioritisation and relative estimates of risk, as well as potentially critical data gaps in ecotoxicity benchmarks for chemicals in wastewater. We use the proposed Urban Wastewater Treatment Directive Extended Producer Responsibility program as a case study to address the potential practical implications arising from scientific methodological choices.

### **3.18.P-Mo287 Impact of Drinking Water Treatment processes on Biocides in the EU**

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The Biocidal Product Regulation (BPR) (EU) 528/2012 regulates the placing on the market and use of biocidal products and ensures a high level of protection for human health and the environment. During the evaluation of active substances (AS) in biocidal products (BP) under BPR, a conclusion on the impact of water treatment processes on residues of AS and their metabolites in water abstracted for the production of drinking water could not be drawn so far. To fill in this information gap, ECHA and EFSA jointly developed a new guidance document “Impact of water treatment processes on residues of active substances or their metabolites in drinking water”, published in August 2023. It aims to provide a clear framework for risk assessors and facilitate risk managers decisions concerning the approval of AS and products authorisation.

The guidance enables the identification of public health concerns from exposure to harmful by-products in drinking water and focuses on water treatment methods commonly used in the EU.

To ensure a proportionate approach and avoid unnecessary testing, a tiered framework is proposed in order to provide information on:

- Water residues that require further assessment;
- New potentially harmful transformation products that may be formed during drinking water treatment; and
- How to conduct a risk assessment that considers consumption of drinking water.

For the biocides sector, this guidance document means that, before they can market new products, manufacturers will be required to demonstrate that the products do not pose a danger to drinking water production.

ECHA proposed that the applicability of this guidance would be only for future applications outside of the review program (RP) or at the renewal of the approval (RNL) (excluding ongoing renewal evaluations). Therefore, the guidance would not apply to the RP substances under evaluation or those currently under RNL procedure. For product authorisations, the guidance is proposed to be applied, only once it has been used in the evaluation of the active substance(s) the product contains.

The presentation will provide details on the implementation of the new drinking water treatment guidance on the assessments for biocidal AS approvals/BP authorisations and discuss consequences and challenges for applicants.

**Keywords:** Biocides, active substance approval, biocidal product, drinking water treatment, chlorination, ozonation, UV disinfection, sand filtration.

### **3.18.P-Mo288 IDEN2REMOVE: Identification and Removal of Site-Specific Organic Pollutants to Preserve the Quality of Water Resources**

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The project IDEN2REMOVE aims at tackling organic pollution in water, which is one of the major causes of water quality impairment. It will deliver tools to uncover site-specific priority pollutants (SSPPs) in the different stages of the urban water cycle, so that preventive and corrective measures can be adopted to reduce or even avoid exposition to these chemicals. The proposed research i) will deliver a low-tech and low-cost active sampler to collect and simultaneously concentrate by solid-phase extraction time-integrated water samples, and ii) will advance current state-of-the-art methodologies for wide-scope screening of organic micropollutant mixtures with high-resolution mass spectrometry, in the sense that will cover the most

polar (and unknown) fraction of these mixtures. Information-rich data will be handled with advanced computing software and chemometric tools to characterize organic micropollutants present in each mixture. Moreover, the work conducted in the framework of this project iii) will provide a prioritization index based on the occurrence and hazard properties of the organic micropollutants detected in the water to identify SSPPs, and iv) will explore advanced treatment technologies currently used in water potabilization and regeneration treatment trains (i.e., ozonation, UV irradiation, membrane filtration, and granular active carbon filtration) to remove SSPPs from water. An additional outcome of the project will be v) the information to end-users (citizens) and stakeholders in the water sector on the research topic addressed by IDEN2REMOVE. The project is built on multi- and inter-disciplinary research, based on the collaboration of experts in environmental analytical chemistry and water treatment technologies. Besides reviewing the main objectives and expected impacts of IDEN2REMOVE, the work presented will introduce the three water circular economy labs where the project will be implemented and will present the first results obtained in its first steps regarding the low-tech and low-cost active water sampling device, and the methodology to be applied for wide-scope characterization of organic micropollutants mixtures in water.

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### **3.18.P-Mo289 An integrated modelling framework for predicting wet-weather discharges pollution**

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Wet-weather discharges from urban areas by combined wastewater systems represent a threat for surface waters: when the system capacity is reached during medium/big rain events, an untreated mixture of stormwater and wastewater is discharged through combined sewer overflows (CSOs) or bypass (BP) of wastewater treatment plants (WWTPs) to a receiving water body. There is currently a lack of knowledge on the actual activation of CSOs and the actual volume and pollution discharged by each system, due to lack of proper monitoring and modelling tools. However, the proposal for the revision of the Urban Wastewater Treatment Directive (UWWTD) proposes to assess the pollution and volumes discharged during wet-weather events and limits the loads discharged to a small percentage of the collected wastewater during dry-weather (1% to 3%). It is clear that to be able to perform this assessment, proper modelling tools are needed.

An integrated modelling framework was applied to a specific case study. The system considers not only the sewer system with all CSOs, but also the WWTP with the bypass. Data available on hydraulics of the sewer system were limited to few flowmeters and info on activation and deactivation of CSOs in time. Many information were present on the WWTP and on the bypass, including flowrate. Monitoring campaigns were then performed at the WWTP, both in wet-weather and in dry-weather. Moreover, samples were collected of the WWTP bypass at different timeframes for 7 events in 2023.

A conceptual model of the system was developed and implemented in WEST (DHI A/S). Both the hydraulic model and the quality model were calibrated in dry-weather and in wet-weather. It was possible to predict the activations of different CSOs structures, the volumes and the pollution discharged. Moreover, a comparison between the loads contribution of CSOs, WWTP bypass, and effluent of the WWTP was performed, together with a comprehensive risk assessment.

This study will allow for a comprehensive understanding of the loads discharged by an entire catchment, including combined sewer system, CSOs, WWTP bypass, and WWTP effluent in dry- and wet-weather conditions. Based on this findings, proper interventions on the system can be planned and the efficacy of the implementation can be evaluated in advance.

### **3.18.P-Mo290 Evaluating the Occurrence of Chemicals of Emerging Concern in Tomato Plants: A Field Study on Agricultural Wastewater Reuse**

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With increasing global concerns over water scarcity, there is growing emphasis on employing sustainable agricultural practices, such as wastewater reuse. However, the utilisation of treated wastewater can potentially introduce chemicals of emerging concern (CEC) into the environment. These chemicals can then be taken up into the edible parts of plants, entering the food chain and posing a potential risk to human health. This study investigates the safety of employing treated wastewater in tomato cultivation, focusing on the uptake of 27 CEC from different categories, through three specific aims: (1) to evaluate the uptake of CEC in tomatoes grown in soil and soilless media; (2) to study the translocation of CEC in plants; and (3) to assess dietary exposure to CEC from consumption of wastewater irrigated tomatoes. Our experiments involve hydroponic and lysimeter experiments, each consisting of four distinct growing regimes: potable water, potable water with the addition of CEC (1 mg/L), treated wastewater, and treated wastewater with the addition of CEC (1 mg/L). Aqueous and plant samples were extracted with validated methods based on solid phase extraction (Prime HLB 96 well plate 30 mg: aqueous samples) and ultrasound extraction (3 cycles, 20 minutes) followed by centrifugation (9000 rcf, 20 minutes) and solid phase extraction

(Prime HLB 500 mg: tomato samples) and extracts were analysed using liquid chromatography-tandem mass spectrometry. In hydroponically grown tomatoes irrigated with spiked media, 11 compounds were identified above the limit of quantification when using spiked potable water and 12 compounds in tomatoes irrigated with spiked treated wastewater. There was lower uptake in soil experiments, with only 4 compounds above the limit of quantification in tomatoes irrigated with spiked potable water and 8 in tomatoes irrigated with spiked treated wastewater. The results will be used for risk assessment and understanding the translocation of potentially toxic organic compounds when applying wastewater reuse in agriculture. Finally, this study will add to the broader conversation about the safety of consuming crops grown using treated wastewater. With water resources becoming increasingly scarce, these findings underscore the critical significance of responsible wastewater management in safeguarding our food supply and advancing sustainable agricultural practices.

### **3.18.P-Mo291 Biocide Contamination of Domestic Greywater: an Indicator of Uses by Inhabitants**

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Biocidal substances are used in several areas of the domestic environment (pharmaceuticals and personal care products, pesticides, cleaning products, etc.) but they can be a threat to environmental and human health. It is therefore essential to characterize contamination by biocidal substances in domestic greywater, which is too poorly documented. This will enable us to estimate the contribution of domestic activities to the biocidal substance discharges into the receiving environment. The aim of this research is (i) to conduct a survey on inhabitant habits in terms of product used and practices; (ii) to characterize domestic greywater contamination by analysing 29 prioritized biocidal substances; and (iii) to compare both approaches in order to assess the effect of inhabitant habits on greywater contamination. To carry out this study, greywater samples were taken from seven dwellings in the Paris conurbation (four washing machine samples, six manual dishwashing samples, five floor cleaning samples, four shower samples and one sink sample). 29 biocidal substances were quantified by liquid chromatography and tandem mass spectrometry. Information about the formulation of products used by participants was collected in order to establish a link between uses and contamination. 3 investigations were conducted in supermarkets to complete information about formulation and improve knowledge on marketed products. 26 molecules were quantified in at least one of the 20 samples. Four substances have a quantification frequency higher than 75%: 3 quaternary ammonium compounds (QACs) and 1 isothiazolinone. The substances quantified vary from one dwelling to another, but much more so according to the type of greywater used. Floor greywaters are the most concentrated when manual dishwashing and shower are the less concentrated. The highest concentrations were measured for QACs (benzalkonium chloride C16 > 20,000 ng/L) in floor greywater. Isothiazolinone and QAC concentrations are highest in greywater in line with the results of the market and inhabitant's surveys, which highlights the predominance of these molecules. The maximum concentration of cypermethrin measured exceeds 6,000 ng/L and can be explained by the presence of carpeting in corridors outside the dwelling. This new database gives us an idea of biocidal contamination in dwellings. The final aim is to estimate the daily tonnages of biocidal substances emitted from domestic environments via greywater.

### **3.18.P-Mo292 Advancing Technologies to Minimize Micro- and Nano-plastics (MNPs) in Industrial Laundry Wastewater from Bisperbjerg Hospital (Copenhagen, Denmark).**

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Washing textiles can release millions of microfibers into the water. Of these, more than half are estimated to be synthetic and eventually transform into MNPs (-micro, -nanoplastics) in the aquatic environment. Once in WWTPs, wastewater undergoes different filtration processes that capture MPs, among other hazardous materials. The efficiency of these processes is remarkably high, however, the volume of water filtered by these facilities daily and worldwide is so high that, still, massive amounts of MNPs are released altogether into the environment. Additionally, current technologies do not target sub-micron sized particles, hence, nanoplastics are expected to cross these membranes.

Targeting hotspots can be useful for testing new technologies that would allow for better water depuration. Previous studies have demonstrated that washing of textiles generate large amounts of microplastic fibres (Cai et al. 2020; Luogo et al. 2022), thereby constituting a potential hotspot for release. In this study, we use the Bisperbjerg Hospital (Copenhagen, Denmark) laundry facilities as a case study to identify and measure the amount of MNPs that are emitted with the laundry effluent. This industrial-scale laundry is used for the disinfection and washing of all the textiles used in the hospital, hence, amounts of MNPs released daily are expected to be high. In a subsequent phase of the SusBrane project (susbrane.dtu.dk), this laundry will be used as a pilot test for development of new ceramic membrane technologies that will specifically target MNPs, with the aim of achieving sufficient water quality so this water could be reused by the hospital laundry, saving energy and water resources.

For this purpose, laundry wastewater will be analyzed before and after the newly developed membranes. Water samples will be collected in every step of the laundry process and filtered under vacuum to extract present MNPs. The obtained MNPs will be later analyzed by laser-direct infrared (LDIR), a semi-automatic technology that will re-count the present particles and identify the polymers.

The findings of the Susbrane project should ultimately allow the implementation of more efficient filtration membranes in full scale, reducing an important source of MNPs to the wastewater in the Copenhagen area. Additionally, the increased volume of



reusable water in the hospital laundry facilities will contribute to save energy and enable more efficient water resource consumption.

### **3.18.P-Mo293 Membrane Distillation as Technology to Remove Microplastics in Drinking Water Production by Desalination: Lab-scale System Performance, Microplastics Behavior and Removal, and Future Research**

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The study of the occurrence and fate of microplastics (MPs) through the water supply chain has been identified by the World Health Organization as a priority research need since MPs are classified as contaminants of emerging concern with potential adverse effects on human health. There is also a need to ensure drinking water safety and affordability, and to minimize the effects of the increasing frequency of extreme events, such as droughts and heat waves, on freshwater availability. Membrane Distillation (MD) is a thermally driven separation process employed to obtain water from wastewater or seawater, with a high rejection efficiency for undesired non-volatile substances and particulate matter. Thus, it can be used as a viable technology to achieve vital progress to counter the growing drinking water crisis. The main goals of this work comprised the i) assessment of MPs behavior and removal during a lab-scale treatment of seawater with MD, and ii) the potential impact of the presence of MPs in the MD process regarding the amount of water produced and its quality. For that, i) filtered seawater with known spiked amounts of MPs of unplasticized poly(vinyl chloride) (UPVC) was fed into the MD system, ii) the MD parameters (vapor pressure gradient, membrane permeability for water, and the interval permeate flux) were monitored during the experiments, iii) the quality of the treated water was assessed in terms of its conductivity, salinity and presence of MPs (> 1.2  $\mu\text{m}$  by Raman microscopy), iv) the MPs were recovered and characterized to assess their potential aging during the treatment, v) the membrane used in each experiment was characterized. The data collected revealed that a high load of MPs (> 0.1  $\text{g L}^{-1}$  of UPVC) can decrease the amount of treated water produced. However, under the expected loads found in the environment, the presence of MPs that are relatively resistant to temperature is expected to have minimal interference with the normal operation of a desalination unit based on MD. The treated water quality was good concerning conductivity (maximum 10.6  $\mu\text{S cm}^{-1}$ ) and salinity (0.0 ppt), with no signs of the spiked MPs being found in it. At the end of the experiments, MPs were recovered from the system, suggesting a very high removal efficiency with MD ( $\geq 99\%$ ). This constitutes one of the first assessments of this technology to remove MPs during desalination, with favorable results, and raises new research questions to be tackled in the future.

### **3.18.P-Mo294 Do the Treatment Units Fragmentate or Remove Microplastics? A Case Study of an Urban Wastewater Treatment Plant (Southeast of Spain)**

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Plastic enters the environment in different ways, such as through wastewater treatment plants (WWTPs), which play an important role in releasing microplastics (MPs) into the environment. MPs are highly persistent and bioaccumulated in living organisms, suffering multiple adverse effects from their interaction with the MPs and their associated additives and contaminants.

This research aims to study the performance of various technologies in municipal wastewater treatment for microplastics removal and to understand the fate of microplastics through a wastewater surveillance study.

Four sampling campaigns were carried out during the four seasons of the year 2023 (from January to November), in an urban WWTP in south-eastern Spain with a daily water discharge of 7,500  $\text{m}^3/\text{day}$ . The sampling points were selected according to the different treatment stages. All the samples were separated by size using a set of sieves ranging from 25 to 1000  $\mu\text{m}$ . Depending on the characteristics of the samples, they were treated with Fenton/peroxide oxidation, enzymatic digestion, and/or density separation to remove organic matter and isolate the microplastics. Visual sorting with a stereoscopic microscope (10-80x) was for MP identification and quantification. MP identity was confirmed using  $\mu\text{FTIR}$ .

The wastewater analyzed during the first sampling campaign showed a concentration of 435 MPs/L in the influent and 23 MPs/L in the effluent, reaching a 95% MP removal efficiency. The treatment units in the water line showed different behaviors: 1) during the pre-treatment, after the grit and grease removal unit, a concentration of 431 MPs/L was found; 2) after the biological treatment, the presence of MPs increased to 1,323 MPs/L; 3) after the secondary clarifier, the removal efficiency was 95% (24 MPs/L). 246,595 MPs/kg wet weight were found in the dewatered sludge (83% moisture). In all the treatment units, the MPs with sizes between 100-500  $\mu\text{m}$  were predominant (41-65%), and fibers were the most prevalent type of MPs (65-80%). The MPs analyzed are made of polyurethane, polyethylene, polypropylene, polyester, polyacrylonitrile, synthetic cellulose, etc. Polyurethane fragmentation was observed during pretreatment, and secondary treatment.

The MPs emission through the water effluent is approximately  $1.72 \times 10^8$  MPs per day. An improvement of the pretreatment, primary treatment, and biological reactor operative conditions can lead to a higher MP removal efficiency.

### 3.18.P-Mo295 Development of an analytical method for the simultaneous determination of 50 semi-volatile organic contaminants in wastewaters

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The amount of chemicals released into the environment have been a matter of study, particularly since the explosive growth of the chemical industry. Recent decades have witnessed a shift in focus towards studying the so-called contaminants of emerging concern (CECs) due to their potential ecotoxicological impacts, including habitat loss, reduction of biodiversity or accumulation within various trophic levels. CECs constitute a heterogeneous group of compounds with varying physicochemical properties, including semi-volatile organic compounds (SVOCs) such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phthalate esters, organochlorine pesticides, and other environmental endocrine disruptors. While the analysis of polar compounds in aqueous samples is widespread, the trend of SVOCs accumulating in biological tissues and different environmental compartments underscores the importance of their comprehensive monitoring. This work aimed to study the feasibility of extending a previously validated methodology for the analysis of polar compounds in aqueous samples to include non-polar compounds within a harmonised analytical procedure using gas chromatography coupled to mass spectrometry (GC-MS) for the chemical analysis. A rigorous evaluation of the factors influencing compounds' preconcentration during solid-phase extraction (SPE) was performed, covering the elution and evaporation steps, to improve the extraction yield. For the extraction step, the hexane:toluene mixture (4:1, v/v) was selected as the optimal elution solvent, showing recoveries ranging from 27 % to 92 %. Significantly, the addition of 5 % of MeOH before extraction was crucial for the enhancement of the recoveries. Concerning to the evaporation step, the addition of isoctane (approximately 0.5 mL) before, during and when around 0.5 mL of the extracts remained in the test tubes, seemed to be beneficial resulting in recovery values within the range of 40 % to 130 % for most of the compounds. Overall, the developed methodology exhibited robust performance for the simultaneous determination of 50 SVOCs and was successfully applied for the analysis of real wastewater treatment plant (WWTP) effluent samples. The multitargeted GC-MS analysis revealed the presence of 15 compounds, with lindane and its analogue beta-hexachlorocyclohexane, bis(2-ethylhexyl) phthalate and phenanthrene the most prominently detected at elevated concentration levels (41 – 18000 ng/L).

### 3.18.P-Mo296 In vitro toxicity of road runoff from different road types using reporter-gene assays

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Rapid urbanization and the rising number of motor vehicles over the past few decades have resulted in dramatic environmental changes. In particular, tire and road wear particles (TRWPs) in road runoff are of increasing concern. Nonetheless, the environmental fate and ecotoxicological effects of road runoff are yet to be understood. Furthermore, the possibilities of interactions between TRWPs and other road runoff pollutants are rarely known. The presented study will be carried out as part of the interdisciplinary RoadTox project, which aims to conduct an ecotoxicological risk assessment of road runoff and further prioritize measures for input mitigation.

Runoff samples were taken throughout the years 2022 to 2023 at three different model road sites (highway, sub-urban and urban road) in Aachen, Germany, covering seasonal and meteorological variability (e.g., dry periods, freezing conditions, heavy rain events). Subsequently, the water samples were filtered and extracted via Solid Phase Extraction (SPE). In addition, organic extracts were prepared from suspended particulate matter (SPM), which were isolated from corresponding runoff samples and freeze dried prior to Ultrasound-Assisted Extraction (UAE). For use in *in vitro* assays, aliquots of the extracts were transferred to DMSO. An *in vitro* biotest battery with several reporter gene assays (dioxin-like activity, (anti-)estrogenic, and anti-androgenic activity) was applied to assess the mechanism-specific toxicity. Prior to the measurement of the mechanism-specific endpoints, the neutral red assay was performed for each used cell line to determine the cytotoxicity.

In previous *in vitro* studies, endocrine disruption and dioxin-like activities were already detected in selected samples. For the samples used in the current study, extensive *in vivo* data obtained from fish embryo toxicity assays and chemical data are available. Hence, the aim of the presented study is to provide corresponding mechanism-specific *in vitro* data focusing on the

comparison of different road types. The study is still in process, and the data obtained will be available for the presentation. The results will contribute to a better understanding of road runoff ecotoxicity and to identify possible toxicity drivers.

The project is funded by the Ministry for Environment, Agriculture, Conservation and Consumer Protection of the State of North Rhine-Westphalia (MULNV) and the RobustNature network, Germany.

### **3.18.P-Mo297 Elucidating the Inhibitory Effects of Phenolic Moieties in Natural Organic Matter on the Photodegradation of Organic Micropollutant**

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UV-induced photochemical transformation efficiently degrades organic micropollutants (OMPs) without requiring a catalyst. This process involves the photo-dissociation of O<sub>2</sub> and self-sensitized pollutants, generating photochemically produced reactive intermediates (PPRIs) including hydroxyl radical (HO<sup>•</sup>), singlet oxygen (<sup>1</sup>O<sub>2</sub>) and excited triplet state. However, the diversity and complexity of water matrices, particularly the presence of natural organic matter (NOM), poses challenges to photochemical transformation of NOM. NOM can absorb UV light, scavenge the radicals/oxidants or enhance the reactive species formation, altering pathways for OMPs elimination.

Natural organic matter is a complex mixture of heterogenous compounds with various functional groups and molecular sizes, including aliphatic chains linked to substituted aromatic rings and attached functional groups like amides, carboxyl, hydroxyl, amine, and others. Notably, NOM features phenolic moieties (mono- or polyhydroxylated benzene units) known for their intriguing electron-donating, antioxidant properties in oxidation reactions. However, the specific role of these phenolic moieties in the formation of PPRIs, and their potential inhibitory effects in photolysis remains uncertain, thus establishing the focus of this study. To investigate this, Tannic acid (TA), gallic acid (GA), and catechin (CAT) were selected as representative surrogates for NOM containing phenolic moieties. Their impact on the photochemical transformation process was evaluated and compared with widely used Suwannee River NOM (SRNOM). Atrazine (ATZ) was selected as the probe OMP due to its susceptibility to photolysis and established photolytic kinetics data. In this investigation, a significantly higher concentration of HO<sup>•</sup> was observed compared to <sup>1</sup>O<sub>2</sub>, and the triplet excited state (<sup>3</sup>NOM\*). This finding suggests that the substituted phenols, particularly those with carboxylate-substitutions, played a substantial role in the formation of HO<sup>•</sup>. However, the inhibitory effects induced by these phenolic moieties were significant, mainly attributed to the direct photolysis inhibition caused by the inner filter effect. When compared with SRNOM, similar trends with less pronounced formation of PPRIs and inner filter effects were observed. Therefore, this study elucidates the role of phenolic moieties on the photochemical transformations, thereby deepening our understanding of the fate of OMPs in natural water.

### **3.18.P-Mo298 Comparison of nanofiltration and reverse osmosis membrane in the removal of pharmaceuticals from water**

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Presence of pharmaceuticals in water can have negative impact on human health, as well as the aquatic environment. Since pharmaceuticals are continuously introduced to water systems and conventional water treatments are not efficient enough in their removal, additional processes for their removal from water are required. Membrane separation processes, such as nanofiltration and reverse osmosis, could be efficient in the removal of pharmaceuticals from aquatic environment. The aim of this study was to compare the efficiency of nanofiltration and reverse osmosis membrane in the removal of four pharmaceuticals, acetaminophen, salbutamol, carbamazepine and bezafibrate, using dead end filtration equipment. Two commercially available polyamide membranes, nanofiltration membrane with molecular weight cut off 150-300 Da and reverse osmosis membrane with molecular weight cut off 100 Da, were used for the removal of pharmaceuticals from a model solution. Efficiency of nanofiltration membrane in the removal of acetaminophen, salbutamol, carbamazepine and bezafibrate was 51.59%, 84.95%, 92.79% and 91.05%, respectively. However, higher removal efficiencies were observed with reverse osmosis membrane, with values of 92.71%, 86.29%, 96.53% and 94.99% for acetaminophen, salbutamol, carbamazepine and bezafibrate, respectively. Lower rejection rates of selected pharmaceuticals with nanofiltration membrane are due to the higher molecular weight cut off of the membrane. The greatest difference in rejection rates between nanofiltration and reverse osmosis membrane was observed for acetaminophen, due to the lowest molecular weight (151.16 Da). Salbutamol, carbamazepine and bezafibrate have higher molecular weights and therefore, the differences in the removal efficiencies between nanofiltration and reverse osmosis membrane were lower. In conclusion, reverse osmosis membrane was more efficient in the removal of selected pharmaceutical, however, nanofiltration membrane also had high removal efficiencies for pharmaceuticals with higher molecular weights.

### **3.18.P-Mo299 Effect of carbon nanoparticles ozonation on their properties and affinity to emerging contaminants**

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The release of nanomaterials (ENMs) to the environment can occur during the use of materials containing nanoparticles, their

processing, disposal and storage. It is assumed that the main part of ENMs enters the environment with wastewater. During transport, ENMs may undergo various transformations, such as: oxidation and reduction, dissolution, adsorption, biotransformation, aggregation and deposition. These transformations can lead to changes in ENMs properties. Since ENMs first end up in the wastewater, it can be expected that they will undergo various transformations in the wastewater. Currently, there is insufficient knowledge about the type and extent of ENMs transformations during water and wastewater treatment. The impact of transformations that ENMs may undergo on their transport and toxicity is also unknown. Ozonation is currently one of the most commonly used methods of water and wastewater treatment. It can be expected that the properties of ENMs will change during ozonation. The main objective of this study was therefore to assess the changes that ENMs undergo under the influence of ozonation and how these changes affected the interaction of ENMs with pollutants. The effect of ozonation was tested on the following nanomaterials: graphene nanoflakes (hydrophilic, hydrophobic, neutral), carbon nanotubes (non-functionalized and functionalized), carbon nanotubes and graphene nanoflakes coated with Zn. Physical and chemical characterization included: elemental analysis (C, H, N), surface area assessment (SBET, pore volume, micropore volume and surface area, and mean pore width), FTIR spectroscopy and Raman spectroscopy. The adsorption affinity of ozonated and non-ozonated materials for triclosan was tested. It was noticed that the physico-chemical properties of ENMs changed after ozonation. These changes depended on the type of ENMs. As a result of ozonation, there was a change in the surface properties, especially of nanocomposites. Adsorption depended on the type of ENMs. Overall, however, ozonation decreased the adsorption affinity for most of the tested ENMs.

### **3.18.P-Mo300 Ozonation for wastewater treatment in water REUSE: organic matter reduction, micropollutants degradation and transformation products identification**

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Water, a vital resource for the environment, human well-being, and economic development, is increasingly threatened by anthropic activities. Wastewater (WW) reuse after adapted treatment represents one of the existing solutions to decrease tension over water resources. However, it is associated with risks caused by the presence of micropollutants and pathogens. Ozonation is then considered as a potential tertiary WW treatment for the elimination of these pollutants. The aim of this study was to determine the effectiveness of ozonation as a cost-effective tertiary treatment for the removal of emerging contaminants and organic matter in the context of WW reuse. We also studied the formation of transformation products (TPs) by Non-Target Analysis (NTA).

Ozonation experiments were performed in a 3 L ozonation pilot glass stirred semibatch reactor ( $V_{\text{reactor}} = 3 \text{ L}$ , 250 rpm) with TWW sampled from Clairia wastewater treatment plant (WWTP). The total gas flow was  $60 \text{ NL}\cdot\text{h}^{-1}$  and the inlet ozone gas concentration ( $[\text{O}_3]_{\text{G,inlet}}$ ) was  $5 \pm 0.2 \text{ g}\cdot\text{Nm}^{-3}$ . Water quality analysis were conducted based on global parameters such as Chemical Oxygen Demand (COD) and  $\text{UV}_{254 \text{ nm}}$  absorbance... Target analyses and NTA were carried out using High Performance Liquid Chromatography coupled to a High-Resolution Mass Spectrometer (HPLC-HRMS) to follow the elimination of eight pharmaceuticals and two pesticides (native concentrations), and the formation of TPs in the treated wastewater (TWW).

Ozonation improved the water quality by effectively degrading the effluent organic matter (OM). COD decreased up to 25 %.  $\text{UV}_{254 \text{ nm}}$  absorbance decreased steadily between 0 and  $0.4 \text{ mg}_{\text{O}_3}\cdot\text{mg}_\text{C}^{-1}$ , where it reached a minimum value of 0.04. These results show that ozone oxidated OM and thus reduced the wastewater organic content. Finally, the decrease in OM aromaticity, as demonstrated by  $\text{UV}_{254 \text{ nm}}$  absorbance reduction, showed that ozonation modified OM by generating simpler molecular structures.

Micropollutants degradation was significant even at low ozone doses. Hence, the elimination was superior to 80 % for nine out of ten quantified compounds at an ozone dose of  $0.074 \text{ mg}_{\text{O}_3}\cdot\text{mg}_\text{C}^{-1}$  (corresponding to 30 minutes of ozonation). However, micropollutants oxidation generated TPs that may be toxic. Some of these TPs have been identified using NTA, leading to a better understanding of the ozone process and its implications for WW reuse.

### **3.18.P-Mo301 A Nature-based Solution to Minimize Reverse Osmosis Concentrate Toxicity**

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Reverse osmosis (RO) offers an excellent way to remove a plethora of recalcitrant contaminants from aqueous matrices such as drinking water. However, a substantial drawback is the produced waste stream, the RO concentrate. As the concentrate consists of the filtered pollutants, such as micropollutants or bacteria, its disposal has to be carried out in a safe and sustainable manner. Frequently, the concentrate is discharged into the open sea or into surface waters. Especially in water bodies with small volumes, the impact can be substantial with potential ecotoxicological effects. Hence, the handling and management of RO concentrate may benefit from cost-effective and sustainable remediation techniques. Therefore, this work presents a nature-based solution to treat RO concentrate with soil retention filters (RSF) to minimize its potential toxicity before discharging it into the environment.

RO concentrate was sampled from a drinking water facility in the Netherlands and was treated by RSF in the laboratory. The filters consisted of gravel and several layers of either sand and calcium carbonate or sand and biochar. The ecotoxicity of the RO concentrate before and after RSF treatment was studied by applying the acute toxicity daphnia assay, performed according to the OECD guideline 202 with *Daphnia magna*. Furthermore, pH, dissolved oxygen, and electrical conductivity were measured.

Ecotoxicological effects were observed for the untreated RO concentrate, albeit maximum RO concentration did not cause complete daphnid immobility. Treatment by RSF caused a complete effect removal, thus indicating that it is potentially safe to discharge the treated RO concentrate into the aquatic environment. Moreover, electrical conductivity was decreased by a factor of 1.5 and would meet therefore international water reuse guidelines.

This work shows that RSF could be a viable treatment for RO concentrate before it is discharged into the environment. Hence, RSFs offer a new option for water authorities to manage RO waste streams by safely discharging the concentrate. Moreover, the treated concentrate may be reused for green infrastructure irrigation. However, further research is required before any final conclusions can be made. Ecotoxicological effects need to be studied on additional species from several trophic levels including potential chronic effects. Furthermore, chemical target and non-target screening would be required as well as long-term performance of the RSF.

### **3.18.P-Mo302 Enhanced Removal of Stormwater Polar Organic Contaminants In Geomedia-Amended Biofilters**

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Innovative drainage systems like green infrastructure (e.g., biofilters) are increasingly valued, designed, and implemented to mimic natural drainage regimes, maximize stormwater runoff capture and treatment, while enabling infiltration into the subsoil for aquifer recharge. Unfortunately, runoff can carry over 150 organic pollutants from vehicle-related sources, and highly polar contaminants (e.g., persistent, mobile, and toxic substances; in short: PMTs) remain challenging to remove in conventional infrastructure. To overcome treatment limitations, we assessed the transport and removal of vehicle-related pollutants such as 1H-benzotriazole, N'N-diphenylguanidine, and hexamethoxymethylmelamine, utilising batch experiments and laboratory biofilters amended with granulated activated carbon (GAC) and biochar. Experimental breakthrough curves demonstrated geomedia amendments can enhance polar organic contaminants removal during passive infiltration, particularly GAC with faster sorption kinetics and higher sorption capacity than biochar. However, contaminant sorption is subject to kinetic limitations and, thus, is sensitive to infiltration flow rates and hydraulic retention times. We inferred these effects could be intensified by dissolved natural organic matter fouling. A more recent biofiltration design is currently being studied to complement geomedia amendments and enhance polar contaminant treatment, even under intermittent dry/wet cycles, due to its benefits on hydraulic performance. Overall, our research contributes to improving polar organic pollutant removal technologies in environmental applications, and designing climate change-resilient biofilters with enhanced hydraulic and contaminant removal performances.

### **3.18.P-Mo303 Fungal Biomass in Wastewater: a Strategy for Pharmaceutical Removal**

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Efficient removal techniques are urgently needed to remove emerging contaminants (EC), such as pharmaceuticals from wastewater, in order to protect water resources. A major source of pollution of inland waters are effluent discharges from wastewater treatment plants (WWTPs) because the existing treatment processes do not efficiently remove EC. Bioremediation based on white-rot fungi (mycoremediation) is particularly promising in decontamination because of the nonspecific nature of the ligninolytic enzymatic system, which can degrade a wide range of EC. Direct use of a colonized mushroom substrate is one method of employing oxidative enzymes. From a practical perspective, this would be less resource-intensive than downstream processing and purification of the enzymes. Furthermore, locally produced plant biomass, such as wetland biomass, could be used to propagate the fungal mycelium. Another option may be submerged fermentation for production of mycelium directly in wastewater. Thus, this research focuses on developing novel, circular and resource-efficient mycoremediation strategies.

In one study, commercial mushroom spawn of *Pleurotus ostreatus* was used to develop a white-rot fungal pellet in water. Laccase activity could be induced by lignin and over time the added lignin accumulated in the pellet. The pellet was tested for removal of 11 different pharmaceuticals. The reduction of total sum of pharmaceuticals in the water phase ranged between 32-37% after 10 min of exposure. Concentration of the antibiotics azithromycin, erythromycin, sulfamethoxazole and trimethoprim was reduced to the highest extent while fluconazole was unaffected. Accumulation of pharmaceuticals in the solid phase, composed of spawn, hyphae and lignin, was correlated with the  $K_d$  of the compound. The size of the pellets allows harvest of the biomass by coarse filtration and the described concept holds promise as a low-cost and sustainable method for enzyme-based wastewater treatment.

### **3.18.P-Mo304 Capacity of biochars produced by cookstoves to remove pharmaceuticals and personal care products from hospital wastewater**

**Brigitte Mukarunyana**, Chemistry, Umea University, Sweden

In sub-Saharan Africa, agricultural and forestry residues are used in cooking but most of these biomasses are considered wastes and are not properly treated, in addition, carbonized materials resulting from cooking are often not valorized. The production of carbonized materials such as biochars may be a sustainable way of managing wastes given that they are renewable sources and their applications are various including energy production, soil amendment and water treatments. To treat wastewater, most hospitals apply conventional techniques that consume a lot of energy to provide oxygen to the treatment system, moreover, they were reported not efficient in removing pollutants like pharmaceuticals and personal care products (PPCPs). In response to that, adsorption has been applied and presented satisfactory results, especially with activated carbon. However, the latter was also reported as not cost-effective and thus became challenging to apply in developing countries. Biochars are therefore prime candidates with high potential for application in low-income countries since feedstock could be locally sourced, easy to produce and environmentally friendly. The study aimed to investigate the capacity of biochars produced by cookstoves in removing PPCPs from real hospital wastewater.

Wastewater samples were collected in one of the largest hospitals in Rwanda and were analysed before and after adsorption experiments on biochars using the LC-MS/MS method. The characterization of biochars produced by 3 different cookstoves using 3 feedstocks has been carried out using DRIFTS, Raman, XPS and BET. In total 28 PPCPs were detected and quantified. The averages of removal rates for all 9 biochars ranged between 14.2 and 65.5% Results from characterization, concentrations of detected PPCPs and the removal rates of biochars will be presented. Findings will contribute to the sustainable management of waste especially in countries with low income and also to increasing databases related to biochars and adsorption studies.

In conclusion, biochars produced by cookstoves removed PPCPs at different degrees, some were more efficient than others, and more biochar characterizations are needed to clarify what main parameters are involved in the removal of PPCPs.

Reference: Tong Y, McNamara PJ, Mayer BK. 2019. Adsorption of organic micropollutants onto biochar: a review of relevant kinetics, mechanisms and equilibrium. *Environmental Science: Water Research & Technology* 5:821-836

### **3.18.P-Mo305 Identification of Cetirizine Metabolites in a Laboratory-scale Moving Bed Bioreactor System**

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Cetirizine, a typical antihistamine, is barely removed from conventional wastewater treatment plants (WWTPs). Biofilm systems such as moving bed biofilm reactor (MBBR) are reported to be effective in removing these persistent micropollutants but no literature regarding it was published. This study aims to investigate how much cetirizine is emitted from WWTPs and whether MBBRs are effective in removing cetirizine via biodegradation.

Removal of cetirizine in conventional wastewater treatment was conducted by comparing in- and effluent wastewater. Additionally, a monitoring of 80 WWTP effluents from multiple EU countries was conducted. Laboratory-scale degradation tests were performed in Erlenmeyer flasks with AnoxKaldnes™ Z400 carriers. Un-spiked effluent wastewater containing cetirizine was used for the biodegradation kinetic test while the cetirizine metabolite identification experiment was conducted with spiked solutions. Changes in the metabolite concentration over time were observed to deduce the transformation pathways. All measurements were conducted using high performance liquid chromatography followed by high resolution mass spectrometry.

Cetirizine in effluent wastewater ranged between 1 to 10 µg/L in EU countries (found in 114 of 120 wastewater samples) and was persistent in a conventional WWTP. In contrast to the conventional wastewater treatment process, MBBR demonstrated its ability to remove cetirizine. Up to 20% cetirizine in un-spiked wastewater effluent was removed in MBBR incubation over 160 h whereas no cetirizine degradation was observed in flasks containing carriers with no biomass. In the meantime, eight metabolites were identified and their concentration increased over time. Cetirizine *N*-oxide was found to be the dominant metabolite in the MBBR incubation and was formed via *N*-oxidation of cetirizine. The presence of cetirizine *N*-oxide was frequently detected in EU countries (found 119 of 120 samples). Bio-transformation is involved in the removal process with MBBR. A possible transformation scheme was proposed to illustrate how cetirizine was transformed to its secondary or tertiary metabolites. The bio-transformation feature of MBBR suggesting biofilm systems could be used as a tertiary step in wastewater treatment system.

### **3.18.P-Mo306 Ecotoxicological mixture risk assessment of 35 pharmaceuticals in wastewater effluents following post-treatment with ozone and/or granulated activated carbon**

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Reducing the risk posed by mixtures of pharmaceuticals is a goal of current initiatives such as the European Green Deal to reduce anthropological environmental impacts. Wastewater effluent typically contains large numbers of active pharmaceutical

ingredients (APIs). For most APIs, existing technology commonly used in many wastewater treatment plants (WWTP) have removal rates below 20%, adding to the toxic burden of receiving waters.

We present an environmental risk assessment of mixtures of 35 APIs in effluent samples from 82 European WWTPs using the concentration addition model, and identify the respective risk-driving APIs. This is then compared to a corresponding mixture risk assessment of effluent samples from the Danish Hillerød WWTP subjected to post-treatment of varying doses of ozone (0.15–1.05 mgO<sub>3</sub>/mg DOC) and/or granulated activated carbon (GAC).

All 82 WWTP effluent samples exceeded risk thresholds by at least a factor of 30, with a median RQ<sub>SUM</sub> of 92.9, highlighting the need for effluent post-treatment. Antibiotics, analgesics and anti-depressants were among the top risk drivers with 99% of the average mixture risk attributable to azithromycin, diclofenac, venlafaxine, clarithromycin and mycophenolic acid. Effluent mixture risk was reduced by ozonation in a concentration-dependent manner, decreasing below threshold levels to a median RQ<sub>SUM</sub> of 0.83 following treatment with 0.65 mgO<sub>3</sub>/mg DOC. Fresh GAC was also effective at reducing the mixture risk, with a median final RQ<sub>SUM</sub> of 0.04, however no additional reduction in API mixture risk was achieved with ozonation plus GAC treatment (median RQ<sub>SUM</sub> = 0.07).

This is the first study to present a risk assessment of pharmaceutical mixtures in effluent comparing “classical” WWTP processes with additional post-treatment with ozone and/or GAC. We demonstrate the need for additional WWTP treatment technologies, and the efficacy of GAC and ozonation in decreasing the risk to the aquatic environment from pharmaceutical mixtures to below acceptable threshold limits.

### **3.18.P-Mo307 Rapid Small Scale Column Tests for the retention of very polar transformation products of commonly used pesticides on granular activated carbon**

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Very polar transformation products rather than parent compounds are currently contaminating ground waters that serve for drinking water production. In Luxembourg, the ban of s-metolachlor and metazachlor in drinking water protection zones, whose transformation products are widely present in ground waters at levels beyond 100 ng/L, has led to a switch to alternatives that pose hardly less risk for the resources. Drinking water suppliers have started to employ granular activated coal treatment to mitigate the pollution without knowing the efficiency of the coals to retain these polar compounds. As several candidates have been identified that will pose problems in a near future by leaching to the water table it was urgent to evaluate their behavior on activated coal columns and to test different types of coal material. A Rapid Small Scale Column Test (RSSCT) device was established with two parallel lines driven by HPLC pumps and a large fraction collector. Seven different coals of different origin were tested with tracer compounds like diatrizoic and chlorobenzoic acid to evaluate their general retention capacity. From this set of coals the currently used coals by the drinking water suppliers as well as an additional reference were tested for 26 parent compounds and transformation products that are potentially expected or are currently already polluting ground water resources. The tests lasted for 3 days passing 82 m<sup>3</sup>/kg GAC and showed tenfold differences in breakthrough thresholds for diatrizoic acid between the coals. The very polar transformations products followed their log D at pH 7 with breakthroughs of chlorothalonil RA471811>flufenacet-ESA>metazachlor-OA being the most critical. The comparison to running full-scale columns showed that the latter had breakthroughs that occurred 1.5 to 2.6 times faster than on the RSSCT systems.

### **3.18.P-Mo308 Seasonal monitoring of granular active carbon filter regeneration effects on organic micropollutant removal during drinking water treatment**

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Threats from organic micropollutants (MPs) remaining in drinking water after treatment are becoming more urgent with their increasing environmental concentrations. Drinking water treatment plants (DWTP) commonly implemented granular active carbon (GAC) filters for MP removal improvement. However, the aging and overloading of GAC filters supposedly lead to a drop in water quality.

We performed comprehensive research on the effect of the GAC system on drinking water quality, combining two methods based on liquid chromatography hyphenated with mass spectrometry (LC-MS). The studied technology was a DWTP fed by surface water from a reservoir. We analyzed water samples and passive polarsamplers (POCIS) taken from different treatment points and compared the GAC filter with and without regeneration. The sampling campaign was repeated four times in different seasons for a more detailed view of treatment changes with the age of the GAC. The first part of the analysis was a routine target method covering about 50 MPs of interest and revealing an expected instant treatment improvement with a regenerated GAC filter in the first sampling campaign. The following two campaigns noticed a decreasing trend and growth of the MP concentration passing through the treatment system. Finally, there was no significant difference between the newly regenerated filter and the old one in the last campaign. After this hypothesis confirmation, we evaluated results from non-target analysis gained by high-resolution MS in combined full scan and data-independent acquisition mode. All detectable m/z signals were

assessed and filtered for the final mass list. This list was used for compound identification and the complex evaluation of treatment along the technology line and with the age of the GAC filter.

### **3.18.P-Mo309 Sorption of Persistent, Mobile, and Toxic (PMT) and very Persistent very Mobile (vPvM) substances onto pyrogenic carbonaceous materials**

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Chemical pollution, specifically arising from trace organic contaminants (TOrcs) such as persistent, mobile, and toxic (PMT) and very persistent, very mobile (vPvM) organic chemicals poses a global environmental concern. The escalating urbanization, characterized by increasing impervious surfaces amplifies this issue, leading to the deposition and accumulation of pollutants in stormwater. Although green infrastructure aims to manage urban runoff, its efficacy in removing polar contaminants, especially PMT/vPvM substances remains inadequate. This study evaluates 20 cost-efficient PCMs (including biochars and granulated activated carbon GAC) and a suite of 34 PMT/vPvM substances to study the adsorption process under different experimental conditions. Results indicate GAC as the effective adsorbent due to its physical properties, while MSP700 biochar demonstrates commendable competitiveness with GAC. Furthermore, sorption preferences based on the charges of adsorbates highlight the need for the specialized treatment for the different charge compounds. This study contributed to advancing knowledge for the development of treatment technologies targeting emerging contaminants in urban environments.

### **3.18.P-Mo310 TKI Identification – Occurrence and Fate of Tyrosine Kinase Inhibitors as a Novel Class of Pharmaceutical Residues in Municipal Wastewater and Surface Water in the Netherlands**

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The ever changing composition of contaminants in the aquatic environment poses a risk to water quality of surface or groundwater used in the production of drinking water. Mixtures of contaminants with the same mode of action can cause adverse effects which would remain undetected when considering single contaminants. To ensure safe drinking water, it is important to detect and identify potentially hazardous (mixtures of) chemicals (i.e. (novel) chemicals of emerging concern (CECs)) early on. Tyrosine kinase inhibitors (TKIs) are cytostatic drugs used in the treatment of cancers where (non) receptor tyrosine kinases (mediators of cell proliferation, migration and cell cycle progression) act as driver. First marketed in 2001, there are currently over 40 TKIs prescribed in the Netherlands, with more TKIs under development. Despite their ubiquitous use, the impact of environmental TKIs on ecology and human health has not been investigated. The accurate risk assessment of environmental TKIs depends on insight into the presence, fate and composition of the mixture of bioactive TKI residues which include (human) metabolites and breakdown/transformation products (TPs). To determine the introduction of TKIs into surface water via sewage, fourteen TKIs in the influent, effluent, and receiving surface water up/downstream from the effluent discharge points of three municipal wastewater treatment plants in the Netherlands will be quantified by LC-HRMS. Samples from locations with high concentrations of TKIs will be selected for the subsequent identification of unknown bioactive TKI TPs and metabolites, later in the project, using high-throughput effect-directed analysis (HT-EDA) expanded with newly developed bioassays, suspect screening, and enhanced non-target screening. The measured environmental TKI concentrations will help direct further investigation into the presence and fate of TKIs in the Dutch (drinking) water cycle, enable toxicological testing of the mixture and eventually contribute to a more accurate risk assessment of TKI residues in the aquatic environment.

### **3.18.P-Mo311 Influence of UV and UV LED disinfection treatment of wastewaters on the fate of micropollutants and water toxicity**

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Water scarcity is a growing issue globally. Wastewater recycling and reuse can help to contribute to societal sustainability but assuring of microbiological and chemical safety of the recycled wastewater is of premier importance. In the present study, we report outcomes of the pilot study at the municipal wastewater treatment plant (WWTP) that compared innovative UV LED reactors and conventional low pressure Hg-based UV lamp (LP UV) for disinfection of pre-treated effluents. The study confirmed significant inactivation of bacteria by both types of disinfection indicating thus suitability for wastewater reuse. LP UV irradiation also affected concentrations of micropollutants resulting in variable patterns. For example, significant decrease of concentrations was observed for diclofenac, sulfamethoxazole or ciprofloxacin. On the other hand, for some micropollutants such as acetaminophen we observed increased concentration after UV irradiation. Moreover, overall estrogenicity and androgenicity of the water (measured by reporter gene in vitro assays) was observed after both LP UV and UV LED irradiations. Analysis of treated water showed release of micropollutants from their conjugated forms, which occur at concentrations comparable with parent compounds but they are rarely studied at WWTPs [1; 2]. The fate of conjugates - glucuronides, sulfates - was also studied in follow-up laboratory experiments. Deconjugation (followed by increases in concentrations of parent micropollutants) as well as parallel degradation of free micropollutant forms were confirmed by LC MS/MS in different time periods. These observations indicate anti-microbial efficiency but also potential risks for water quality of the UV disinfection treatment of effluents.



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References: [1] <http://dx.doi.org/10.1016/j.chemosphere.2017.04.040> [2] <https://doi.org/10.3390/ijms231911913>.

### **3.18.P-Mo312 Tracking and Removal of Organic Micropollutants in the Urban Water Cycle: the Role of Wastewater Treatment Plants**

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Organic micropollutants (OMPs) are the main cause of water quality deterioration. OMPs include not only a huge variety of anthropogenic chemicals, but also the byproducts generated after natural (photolysis and biodegradation) and engineered processes (chemical, biological and disinfection treatments). Therefore, OMP mixtures present in water are very complex and diverse. The chemical diversity of OMP mixtures is related to pollution sources and engineered solutions that impact the water, the physical-chemical properties of the water itself, and meteorological events, among others. To fully characterize OMP mixtures, wide-scope non-targeted analytical strategies are needed. In this sense, advanced analytical techniques based on high resolution mass spectrometry (HRMS) detection in combination with chemometrics reveal as very useful tools for the comprehensive analysis of OMPs in water. As a case study, this methodology has been applied to evaluate the effect that the construction and operation of a wastewater treatment plant has on the water chemical quality of Genil River as it flows through the municipalities of Huetor Tajar and Villanueva de Mesía (Granada, Spain). For this, grab water samples collected along the river stream before and after known locations of non-treated water discharge and the wastewater treatment plant were collected before and after the wastewater treatment plant came into operation. To cover a wide range of chemicals in the analysis water samples were 200x concentrated through lyophilization, and the extracts obtained were analysed by liquid chromatography-HRMS using a quadrupole-time of flight analyser. Differences among samples and OMPs of interest will be identified by chemometrics. This work aims at assessing the effect of the newly built wastewater treatment plant in the water quality of the Genil River, revealing the OMPs that persist the water treatment, and further exploring their environmental risks, as well as their removal with currently used advanced treatments. Results addressing these objectives will be presented.

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### **3.18.P-Mo313 Assessment of Illicit Drugs Use in Seoul, Capital City of South Korea for 21 Days by Wastewater-Based Epidemiology**

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The recent surge in drug-related crimes has become a serious societal concern both domestically and globally. To suppress and prevent drug offenses, it's imperative to understand the prevalence of illegal drug usage. The drug consumption has primarily been monitored through conventional methods such as surveys, seizure quantities, and prescription records, which have limitations in accurately gauging actual drug usage. To address this, Wastewater-Based Epidemiology (WBE) has emerged as an alternative method, widely adopted due to its ability to estimate drug consumption rates by analyzing drug residues in wastewater. While many countries used WBE for monitoring drug usage, previous domestic studies were limited to certain cities, conducted over a short period (within three days), or involved monitoring only for one day nationwide. Furthermore, the absence of previous monitoring studies in Seoul, capital city of South Korea, makes it crucial to monitor drug consumption patterns in the city, especially considering the significant surge in drug-related crimes. Therefore, illegal drug usage was investigated in Seoul, the capital city of South Korea, for 21 days using WBE to assess illicit drug consumption patterns for the first time by region and social status. Out of the 22 compounds, 10 were detected. Among these, Methamphetamine showed a 100% detection rate, with an average consumption rate of 16.25 mg/day/1000 people, the highest among the detected substances. This study showed lower consumption rates than most other countries. Furthermore, Methamphetamine usage was similar to or lower than previous domestic research. Notably, Cocaine and MDMA, previously undetected, were identified in this study. The usage patterns of these illicit drugs did not exhibit specific daily patterns. While some compounds showed slightly higher consumption on weekends compared to weekdays, no statistically significant differences were observed (Mann-Whitney Rank Sum Test,  $p > 0.05$ ). Moreover, Methamphetamine and MDMA displayed stable daily consumption patterns. Methamphetamine and MDMA consumption rates varied significantly by region ( $p < 0.05$ ), correlating positively with average annual income and education levels ( $p < 0.05$ ).

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### **3.18.P-Mo314 Water Monitoring along Railway Tracks in Germany**

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In general, rail transport shows a lower emission of pollutants compared to other modes of transport. However, it has an impact on the environment and especially on natural resources near the tracks, e.g. surface and groundwater bodies. Organic and inorganic substance emissions from today's and past rail operations and associated infrastructure maintenance works, as well as abrasions are of particular importance. The study focuses on released pollutants and their emission into adjacent groundwater, surface water and into the soil. The results are important for closing current knowledge gaps and creating a basis for a comprehensive environmental risk assessment and the derivation of prevention strategies. Five monitoring sites have been set up along the German railway network. Two of these focus on identifying potential impacts and emissions from railway tracks into nearby surface waters. In addition, the pollutant load in seepage water and groundwater (inflow and outflow) can be determined at all monitoring sites. The monitoring design considers both, relevant emissions from vegetation control and constant input from abrasions. Since 2021, water samples have been taken at least twice a year at all locations and analysed using standardized methods. The results show that the railroad operation emits heavy- and semi-metals in particular into the surface water and groundwater. Following vegetation control and the subsequent application of herbicides, glyphosate and its metabolite AMPA, flumioxazine and flazasulfuron were detected in downstream surface waters as well as in seepage water itself. Herbicides were only detected in surface waters if the precipitation occurred less than 10 days after the vegetation control. Other herbicides (e.g. atrazine, bromacil or pelargonic acid) or organic substances were only detected in traces in the soil around the track. The study forms an important part of the official monitoring concept and gains a deeper understanding of the release of pollutants along railway tracks into the surrounding environment. These investigations are an important contribution to the derivation of prevention strategies for track wastewater treatment and to the sustainable development of rail transport as such.

### **3.18.P-Mo315 Estimation of the usage of drugs of concern in the Southern Ontario, Canada, through wastewater-based surveillance**

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Wastewater-Based Surveillance (WBS) is a powerful tool for assessing and monitoring drug consumption within our communities. This approach has played a crucial role in the public health decisions, especially during the COVID-19 epidemic. However, implementing WBS programs face challenges, such as lack of reliable strategies to normalize the mass load per capita as well as the complexity of extracting these compounds from the wastewater.

To overcome these issues, an analytical method combining solid phase extraction (SPE) with liquid chromatography tandem mass spectrometry (LC-MS/MS) was developed to analyze a 19 of drugs of concern. The method achieved limits of detection from 0.7 to 49.3 ng/L for LSD and methamphetamine respectively. The final method was applied to wastewater collected from January 2022 to February 2023 across several wastewater treatment plants in Southern Ontario revealing varied drug concentrations ranging from 20 to 5000 ng/L. This data is important for comparing per capita drug usage and provide objective insights for health policymakers.

### **3.18.PC Next-Generation Urban Water Management: Improved Understanding of the Fate of Micropollutants, Transformation Products, Pathogens, and Antimicrobial Resistance**

### **3.19 Passive Sampling: Analysis, Transport, Fate and Monitoring of Persistent, Mobile and Toxic Substances in the Environment**

#### **3.19.T-01 PASSIVE SAMPLERS FOLLOWED MASS SPECTROMETRIC TECHNIQUES FOR AN EFFICIENT EVALUATION OF HONEY BEE CHEMICAL EXPOSOME**

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Honeybee (*Apis mellifera*) health has been severely impacted by multiple environmental chemical stressors including pesticide exposure, environmental contaminants and other endogenous or exogenous chemicals. Their impact on bee health is therefore a complex multifactorial problem which requires a holistic investigative approach. Many studies have been directed on such but with important limitations related to very limited analytical scopes, not enough sensitivity, or strong thump in the colonies in long term monitoring studies due to the use of live animals or parts of the beehive for the analysis. Furthermore, the need of standardization hinders the comparability of the results in different studies and varying environments.

In this study, a non-biological passive sampler based on Tenax-TA is described: the APIStrip (Adsorb Pesticide In-hive Strip). The use of this non-invasive sampling device is harmless for colony: the human interaction is minimal, and no resources are subtracted from honeybees. Of particular importance is the capability of standardization and the accumulation of chemicals for periods of time (typically 15 days) that facilitates an extensive monitoring at trace concentration levels of both target and non-target chemicals. The extraction of these APIStrips followed by low- and high-resolution mass spectrometry analysis report an

extensive and unmatched data of chemicals present in the colony. Preliminary tests demonstrated the high efficacy, sensitivity, representativeness, and reproducibility of the APIStrip-based sampling.

Our findings raise the interest in using APIStrip as passive sampler followed by mass spectrometry techniques allowing an extensive evaluation of target (such as pesticides) and non-target compounds. This study not only presents a robust analytical methodology utilizing APIStrips for hive environment assessment but also sheds light on the diversity of organic compounds present in beehives across different areas and temporal settings. Both together resets a clear picture of the chemical environmental exposures of bees in the beehive.

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### **3.19.T-02 Identifying persistent, mobile and toxic (PMT), and very persistent and very mobile (vPvM) substances in stormwater systems using passive samplers**

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Urban stormwater runoff from impervious areas has been recognised as a significant contamination source for various organic and inorganic pollutants in aquatic ecosystems. However, data are scarce regarding environmental pollution levels, particularly for persistent, mobile and toxic (PMT), and very persistent and very mobile (vPvM) substances.

For future monitoring programmes to incorporate critical organic substances, a data-driven approach to support the selection of priority substances alongside systematic, transferable monitoring tools is necessary. In the water phase, passive sampling was highlighted as a promising tool for measuring dissolved organic micropollutants, including PMT/vPvM substances.

In this study, we aim to identify PMT/vPvM priority substances that could potentially occur in stormwater systems in a comprehensive literature review and assess the effectiveness of two different passive sampling receiving phases from AttractSPE<sup>®</sup>, hydrophilic-lipophilic balance (HLB) and styrene-divinylbenzene reversed phase sulfonated (SDB-RPS) disks, for the set of identified potential urban stormwater contaminants.

Based on current literature, a list of forty target analytes was compiled. The list of targets, comprising thirty PMT/vPvM substances, was supplemented by ten high-risk (occurrence >50%, risk quotient > 1) stormwater pollutants previously identified in urban areas. In a laboratory-scale experiment, the applicability of passive samplers for stormwater monitoring will be evaluated for the selected substances. In agreement with previous studies on a set of hydrophilic organic contaminants, we hypothesize that both AttractSPE<sup>®</sup> SDB-RPS and HLB disks are well-suited sampling phases for the chosen PMT/vPvM substances. Minor differences in sample uptake are expected due to slight differences in sampler properties, potentially showing slightly higher mass accumulation on the HLB disks.

Our systematic approach to identifying PMT/vPvM substances has revealed 47 substances that are highly likely to occur in urban stormwater and can be quantified using LC-HRMS/MS. As an initial set of target analytes, forty substances could be prioritised when measuring urban stormwater for PMT/vPvM substances.

### **3.19.T-03 Passive sampling calibration for persistent and mobile chemicals**

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Passive sampling techniques have been widely used for the monitoring of contaminants in water bodies, yet advancements in targeting persistent and mobile substances remain limited. This study focused on evaluating the performance of a polar organic chemical integrative sampler (POCIS) investigating analytes from three different chemical classes considered to have persistent and mobile substances. A laboratory calibration using two types of passive sampling HLB discs (HLB 1 and HLB 2) sandwiched between 5 µm pore size stainless steel membranes and POCIS rings were used to measure the uptake rates for ten analytes over a 28-day period. The discs were made of different materials: HLB 1 disc was made of 90 % sorbent and 10 % binding agent and HLB 2 was made of loose HLB sorbent packed between a glass fibre membrane. In the experimental setup, two stainless steel containers, each holding approximately 96 L, were filled to around 80 L capacity. These containers were then spiked with analytes at a concentration of 0.05 ng/mL, encompassing three different classes of contaminants: four per- and poly-fluoroalkyl substances (PFAS), one triazine, and five triazoles. Over the 28-day period, duplicate water samples were taken daily and HLB samplers removed either in triplicate or a singular replicate depending on the passive sampler configuration on days 1, 2, 4, 7, 14 and 28. Extracted samplers and water samples were analysed using UPLC-MS. Results for the benzotriazoles showed higher sampling rates for HLB 1 (137-643 mL/d) compared to HLB 2 (58-232 mL/d), indicating there is a difference between the disc properties on the uptake of contaminants into the sampler. The sampling rates also indicate that the HLB 1 configuration may be more sensitive to water turbulence compared to the HLB 2 configuration. Therefore, understanding and accounting for these differences in disc properties and their influence on uptake dynamics is crucial for accurately monitoring persistent and mobile substances in the aquatic environment.

### **3.19.T-04 Combination of Passive Samplers, Bioassays and Nontargeted Screening as a Tool for a Decision Making**

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Passive samplers were recognized as low-cost tools for obtaining more representative information on water pollution than grab or 24-hour composite samples. Because we cannot detect all chemicals present even in the extract, the bioassays remain the only tool for biological effect evaluation. However, there is a need to identify effect drivers, which can be forwarded to the next decision-making stage. Several approaches combine targeted and nontargeted analysis with bioassays to the effect's explicability of the mixtures. We applied fractionation in 2D to reduce the complexity of the mixture and used affinity clean-up for selective extraction of unknowns of interest.

Commercially accessible AttractSPE@Discs HLB were deployed in WWTP effluent for two weeks. The initial extract was separated at the Arion polar C18 column into 10 fractions. The battery of bioassays was applied to investigate the effect of individual fractions. The active ones were offline fractionated at the Arion phenyl-butyl column and analyzed with bioassays identically to 1D fractions. All 1D separated fractions, 2D separated active fractions, and initial samples were analyzed by a nontargeted acquisition method combining liquid chromatography separation with full scan high-resolution and data-independent MS2 mass spectrometry. Selective affinity-based purification was applied to decrease the complex extract's matrix effect further and improve the detectability of effect drivers with a two-stage nontargeted screening workflow.

The complexity of the mixture was lowered by fractionation from about 4100 features to 2800 features in 1D fractions. This separation also affected the bioassays themselves as extract cytotoxicity was lowered. The AhR effect was found in two consecutive fractions, further fractionated in 2D. The second dimension showed a good recovery of the 1D observed effect. The number of features detected in all 2D active fractions ranged from 975 to 1900 for F7 fractions and 580 to 1450 for F8 fractions, which was unsuitable for effect driver identification.

The affinity purification seems promising as the fraction's complexity level was lowered much more than in the case of fractionation only. The two-stage acquisition workflow allows for highly selective identification of TTR ligands. Applying affinity-based purification as an additional step to fractionation helps reduce the extract's complexity and enables the successful identification of effect drivers in the mixture.

### **3.19.P Passive Sampling: Analysis, Transport, Fate and Monitoring of Persistent, Mobile and Toxic Substances in the Environment**

#### **3.19.P-We190 Investigation of mutagenic aromatic amines in municipal wastewaters using passive sampling**

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Aromatic amines (AAs) represent an environmentally important group of contaminants, including known and suspected mutagens. They enter surface waters from different sources directly from industrial emissions or indirectly as metabolites of pesticides and have the potential to endanger aquatic ecosystems. Municipal wastewaters (WW) present a not yet-investigated potential emission source of AAs. AA from sources in the indoor environments, emitted to municipal WW, may significantly contribute to their presence in receiving water bodies. Our study aimed to test a representative method for monitoring AAs in WW and recipient surface water using a combination of passive sampling (PS) with targeted analysis and suspect screening.

Three PS variants were tested for monitoring AAs in influent and effluent of a municipal wastewater treatment plant (WWTP) serving the city of Brno, Czech Republic. The uptake of AA to HPS was compared with their content in 24-hour composite water samples. The targeted analysis of samples on LC/tandem mass spectrometry included about 50 AAs with potential to cause adverse effects. Further, suspect screening of AAs on samples was performed using LC/high resolution mass spectrometry to contribute to prioritisation for further targeted developments. Finally, unknown AA patterns accumulated in samplers deployed in WWTP influent and effluent will be compared using non-target screening methods in order to evaluate the AAs that could be eliminated/treated and the newly generated AAs during the treatment process as transformation products.

All samplers accumulated a comparable number of AAs in targeted analysis, and integrative uptake was observed up to 28 days in both influent and effluent of the WWTP. For compounds present simultaneously in water and PS in at least 50% of the samples, sampling rate ( $R_s$ ) estimation was performed. Robustness of  $R_s$  was assessed by comparing their calculated values for compounds occurring in both WWTP influent and effluent. Finally, removal efficiency of AAs in the wastewater treatment was assessed using the sampler variant with robust  $R_s$  values. Our study demonstrates the possibility for obtaining coherent

results with passive samplers despite the differences in their construction. The sampler variant fitted with agarose diffusive hydrogel layer with the broadest range of accumulated compounds is especially attractive for suspect screening or non-targeted analysis of aquatic contaminants.

### **3.19.P-We191 Exposure assessment to organophosphate esters, phthalate esters and alternative plasticizers from recycling workers in Colombia using t-shirts as passive samplers**

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Clothing may be an important contributor to our daily exposure to various chemicals. Wearing clothes in polluted places could be a hidden source of potentially harmful substances. Human on daily lives, clothes constantly interact with the environment, accumulating a range of chemicals. These can come from diverse sources such as air pollution, household dust, and other environment sources. Sampling was conducted at the Industrial Park Recycling in Cartagena, Colombia, where there are workers engaged in the plastic recycling activities. T-shirts made from 100% cotton were provided to volunteer workers from this area at the beginning of their workday. From each t-shirt it was cut a piece of 14 × 20 cm<sup>2</sup>, and afterwards sliced into pieces of 1 × 1 cm<sup>2</sup>. Extraction was performed using pressurized liquid extraction (PLE). Hexane:acetone (1:1) was used as the extraction solvent, and two static extractions were performed at 1600 psi and 50 °C, resulting into an extract around 35 mL. The extracts were injected in an online sample purification and analysis method based on turbulent flow chromatography (TFC) coupled to liquid chromatography (LC) and a triple quadrupole (QqQ) tandem mass spectrometry (MS-MS). On this study, concentrations of 16 OPEs, 11 PAEs and 3 alternative plasticizers were measured in cotton T-shirt samples. In this study, 16 out of 20 OPEs analyzed were detected in cotton shirt samples previously worn by recyclers. The total average concentrations of  $\Sigma_{16}$ OPEs were (mean ± SD) 11.0 ± 12.7 ng/(dm<sup>2</sup>\*h), with a median of 2.7 ng/(dm<sup>2</sup>\*h), ranging from 1.06 to 43.4 ng/(dm<sup>2</sup>\*h). The highest detection frequencies (%DF) were found to be ≥97% for five specific OPEs: TEP, TCIPP, TPHP, TBOEP, and TEHP. These five chemicals accounted for 96% of the total OPEs. The total average concentrations of  $\Sigma_{10}$ PAEs were (mean ± SD) 2,087 ± 2,470 ng/(dm<sup>2</sup>\*h), with a median of 1,040 ng/(dm<sup>2</sup>\*h), ranging from 201 to 11,143 ng/(dm<sup>2</sup>\*h). Detection frequencies (%DF) were found to be 100% for six target PAEs: DEP, BBzP, DnOP, DiDP, DiNP, DHexP, and DEHP and for the rest of the phthalates, %DF ranged from 61% to 94%. Three out of ten chemicals were the predominant PAEs. These results showed that clothings are an important source to exposure to these chemicals. DEHP, DiNP, and DiDP were the predominant PEAs and DEHA, ATBC, and DINA were the most abundant Non-PAEs detected. On the other hand, TEP, TCIPP, TEHP, TPHP, and TBOEP were the most predominant OPEs in the T-shirt samples.

### **3.19.P-We192 The IMPART project – a citizen science approach to using passive samplers to monitor for emerging contaminants**

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The Imperial Monitoring using Passive Samplers to Assess River Tributaries (IMPART) project utilises a citizen science approach to monitoring local rivers for contaminants of emerging concern using a miniaturised 3D-printed passive sampler device (3D-PSD). The 3D-PSDs are manufactured from a methacrylate-based resin and hold up to five individual 9 mm sorbent disks. Seventeen citizen scientists were engaged from four community groups across three UK sites - Brent, Norwich, and Sheffield. Utilising their local river knowledge and experience, the citizen scientists were trained to deploy and retrieve the HLB-orientated 3D-PSDs (unaided) in locations of their choosing for seven days during September 2022. Water samples were collected, and field blanks were exposed by the citizen scientists during deployment and retrieval.

In total, 25 passive sampler devices were successfully deployed and retrieved by the citizen scientists and returned to Imperial College London for quantitative analysis with no device losses. Across all sites, 57 unique compounds were quantified on the passive sampler and 35 compounds were common to all three sites. Bisoprolol was quantifiable on the passive samplers across all 25 deployment sites over the range of 0.01 to 2.0 ng disk<sup>-1</sup>. In the water samples, only 20 unique compounds were quantifiable above method LLOQ across all sites, demonstrating the increased sensitivity of passive sampling, even at a miniaturised scale, for the monitoring of contaminants at environmentally relevant concentrations. Across the three sites, the average mass on the passive sampler was 0.9 ± 1.4 ng disk<sup>-1</sup>, 0.5 ± 1.1 ng disk<sup>-1</sup>, and 0.5 ± 0.9 ng disk<sup>-1</sup> for Sheffield, Norwich, and Brent, respectively. Within each site, sources of pollution were clearly identifiable from the 2.5 to 4-fold increase in the total mass of contaminants accumulated onto the 3D-PSD. There were between four and three unique compounds per site and multivariate statistics are being explored to determine if it is possible to classify rivers based on their chemical profile which has been demonstrated in the London tributaries. This work demonstrates the applicability of the 3D-PSD to citizen science and community engagement projects.

### **3.19.P-We193 Polytetrafluoroethylene (PTFE) Membranes for Passive Sampling of Hydrophilic Compounds in Water: a Big Yes**

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Passive sampling techniques are particularly efficient to monitor organic contaminants across various aqueous matrices. One common type of passive samplers, Chemcatchers, utilises a sorbent covered by polyethersulfone (PES) membranes to sample

hydrophilic micropollutants. However, numerous studies have recommended replacing this membrane due to its high analyte sorption capacity, especially for the less hydrophilic compounds.

This research project investigated the potential of polytetrafluoroethylene (PTFE) membranes in Chemcatcher-like passive samplers. Calibration of 44 hydrophilic micropollutants was conducted using this sampler design within a channel-system featuring four distinct water velocities ranging from 5 to 40 cm s<sup>-1</sup>. LC-MS/MS analyses revealed that the sorbent (styrenedivinylbenzene – reverse phase sulfonate, SDB-RPS) accumulated compounds on average more than 2,000 times than the PTFE membrane. This emphasized the suitability of PTFE as a membrane material for hydrophilic micropollutant sampling.

Sampling rates ( $R_S$ ) were determined for each analyte at varying water velocities, ranging from 0.027 L day<sup>-1</sup> to 0.300 L day<sup>-1</sup>. By establishing relationships with the mass transfer coefficient of the water-boundary layer ( $k_w$ ) that depends on hydrodynamics, it became feasible to determine site-specific  $R_S$ . Additionally, the relative contributions of each phase (i.e., water boundary layer, membrane, and sorbent) to mass transfer was determined. Estimations showed that thicker PTFE membranes ( $\geq 100 \mu\text{m}$ ) would completely shift uptake control towards the membrane when the hydrodynamic conditions exceed 20 cm s<sup>-1</sup>, thus enabling the prediction of  $R_S$  for any hydrophilic organic compound without the need for time-intensive calibration experiments in conditions usually encountered in rivers.

In summary, this project significantly enhances the understanding of the role of membrane characteristics and hydrodynamic conditions in passive sampling. This could potentially facilitate the prediction of  $R_S$  for hydrophilic micropollutants, contributing to the advancement of passive sampling techniques for environmental assessment and monitoring.

### **3.19.P-We194 Characterization of Emission Products Formed during Firing of Small Caliber Ammunition**

*Arjan den Otter and Wouter van de Steeg, TNO, Netherlands*

During the firing of ammunition, emission products are formed to which the shooter and the environment will become exposed to. In order to judge which weapon-ammunition combination can best be used in certain situations to reduce the exposure of personnel as much as possible, it is important to know which parameters influence the formation of the emission products and the routes via which the emission products can leave the weapon. This study provides an overview of the various parameters that directly or indirectly influence the formation of emission products. Besides that two types of setups for experimental determination of emission products are described.

In one type of these setups, the emission is measured at the location where typically the head of the shooter is located and analyzed for a range of toxic compounds and for particles. Using this setup several weapon-ammunition combinations, the influence of using a silencer and firing single shots versus a burst of 10 shots were evaluated. Using another type of setup, the weapon can be fired in a confined space. Both during and after firing and homogenization, the emission products can be analyzed. The benefit of this setup is that the influence of parameters like ventilation on the experimental results is limited, facilitating comparison of different weapon-ammunition combinations studies at different locations.

During experiments it was shown that although the firings were identical, large concentration variations were measured between the individual firings, most likely due to variations in the burning process in the weapon or the ventilation flow. When a silencer was used, typically concentrations of emission products were higher than without silencer. Based on concentrations measured during these experiments, the main components of concern were carbon monoxide and fine dust ( $< 1 \mu\text{m}$ ). For ammunition containing lead and antimony, these are components that need specific attention. Nitric oxide could become a substance of concern when shooting for a long time.

It is important that a standardized method for performing emission measurements under controlled conditions is available. This will enable comparison of the different research results and to draw more solid conclusions regarding the influence of various parameters on the formation of emission products. This will contribute to an improvement of the assessment of the risks to which a shooter is exposed to during firing of ammunition.

### **3.19.P-We195 Personal Exposures of Waste Collection Workers to Nitrogen Dioxide and Ozone During Day Time and Night Shift Hours on 24 Different Collection Routes**

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Waste collection workers are potentially exposed to health, environmental and safety risks due to various chemical and biological substances in the waste to be collected. The job descriptions of waste collection workers usually consist of driving truck, collecting and emptying waste containers or sacks and unloading waste at waste receiving facilities. Typical rear loader operations require manual lifting of materials into collection vehicles but a fully automated collection minimizes manual lifting and reduce exposure to contaminants. There is usually one or two more staff in the vehicle usually at the rear loader besides the driver. In this study, personal exposure to gaseous pollutants was determined in 70 employees working in municipal cleaning works, 24 of whom were drivers and 46 of whom were personnel collecting garbage behind the vehicle. Passive samplers were used to measure exposure concentrations of nitrogen dioxide (NO<sub>2</sub>) and ozone (O<sub>3</sub>). The workers carried the passive samplers with armbands on their arms only during working hours. During the personal sampling, the trucks collected waste along 24

different routes. The exposures of the driver and the workers in the back of the truck were compared and the route-dependent variability of the measured pollutants was analysed. When we analyzed the sampling routes separately according to the traffic density (low, medium, high), it was observed that the NO<sub>2</sub> exposure in the two groups was correlated with traffic density with higher concentrations on routes with higher traffic density. The results also showed that the exposure of workers at the rear loader to NO<sub>2</sub> was significantly higher than that of truck drivers ( $P < 0.005$ ). The exposure of the workers was analysed based on the type of garbage collection (waste container, manual collection of garbage bags, both), and a statistical difference was observed ( $P < 0.005$ ). There is a significant difference between the NO<sub>2</sub> exposure concentrations of drivers working in day and night shifts ( $P < 0.005$ ).

Keywords: *Passive sampling, NO<sub>2</sub>, Ozon, Waste collection, Exposure*

### **3.19.P-We196 Characterization of Covalent Organic Frameworks as Adsorbents for Pharmaceuticals in Water**

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In the present work, several covalent organic frameworks (TpBD(CF<sub>3</sub>)<sub>2</sub>, TpBD-Me<sub>2</sub> and TPB-DMTP) were challenged to adsorb several pharmaceuticals considered among the most important emerging pollutants in water: the antibiotics sulfapyridine, sulfamethoxazole and tetracycline and, the anti-inflammatory diclofenac. TpBD(CF<sub>3</sub>)<sub>2</sub> was the best adsorbent for sulfonamides. The adsorption yields reach 70 and 88% for sulfamethoxazole and sulfapyridine respectively, showing rapid adsorption reaching equilibrium at 30 min. The pH plays a significant role in the adsorption mechanism, especially at basic pH where the target molecules are negatively charged, which suggests that there is an effect of repulsion of charges or that the formation of hydrogen bonds is prevented.

For the adsorption of tetracycline, TpBD-Me<sub>2</sub> proved to have the best potential to be used for adsorption as it retained a good adsorption efficiency (up to 90%) while not having to use the fluorinated material. When tested at different pHs, the adsorption of tetracycline by TpBD-Me<sub>2</sub> was more efficient at pH 6 when the pharmaceutical compound was at the dominant zwitterionic form.

For the adsorption of diclofenac, TPB-DMTP showed the highest adsorption capacity between the analysed COFs (up to 50 mg/g experimentally). This COF stands out for its higher surface area and larger pores. The adsorption of diclofenac by TPB-DMTP was more efficient at pH 6 than pH 9, pointing to a lower affinity of the COF for the deprotonated form.

Isopropanol was determined as the best desorption solvent for all pharmaceuticals, with percentages of recoveries between 65% and 100% being sulfapyridine and diclofenac the less and the most efficiently desorbed, respectively. Desorption kinetics indicated that the optimum time of desorption was between 15 min and 2 h depending on the pharmaceutical. This desorption capacity makes reuse possible in up to five successive cycles of adsorption-desorption, enabling the implementation of these materials for passive sampling more sustainable and cost-efficient.

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### **3.19.P-We197 Comparison of Recent Attract HLB to Established Biotage HLB and Empore SDB-RPS Phases for Passive Sampling of Pesticides**

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In recent years, passive sampling (PS) has been widely applied to monitor organic micropollutants in aquatic environments as a cost-effective tool. Its ability to enrich compounds in situ and providing a time-weighted average makes it a suitable alternative to costly automated sampling. Various PS phases have been studied and characterised regarding uptake of pesticides from water. However, with some phases having experienced shortages in production, alternative materials need to be investigated and compared to enable faster changes from one producer to another in case of discontinuation, even more so as there is no standardised material for PS with secured production.

This work investigates the recently introduced hydrophilic-lipophilic balanced (HLB) phase (Attract product line) from the French company Affinisep for uptake suitability of PPPs and compares it to the well-established HLB phase produced by Biotage and the widely applied polystyrene-divinylbenzene reversed-phase sulfonated (SDB-RPS) phase of Empore.

The samplers (47 mm disks, custom steel-plate housing) were calibrated for different hydrodynamic regimes (flowing vs. stagnant, continuous replenishment of spiked water) over a two-week period. In addition, all PS phases were evaluated in the field to compare in-situ calibration with the laboratory study. Furthermore, partition coefficients were determined via a shaking and equilibrating experiment. Additionally, the Affinisep HLB phase was further investigated to test its ability for sediment pore water sampling of PPPs for a future study using custom-made sheets.

Our data suggest that the more recently introduced HLB (Affinisep) phase is well suited to study a wide range of pesticides (69 analytes) with different  $\log K_{ow}$  (-1.7 to 5) compared to the well-established Biotage HLB and Empore SDB-RPS phase. Our kinetic uptake studies show that Affinisep HLB exhibits higher sampling rates compared to HLB from Biotage, resulting in larger analyte amounts sorbed to the disk overcomes issues of sensitivity. The data indicates the suitability for short-term deployments of this new PS phase.

Overall, this study provides new PS properties such as sampling rates and partition coefficients for a wide range of PPPs which will help improve existing uptake models.

### **3.19.P-We198 Accelerated solvent extraction (ASE) of passive samplers as an efficient tool for the monitoring of hydrophobic micropollutants in water**

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Water, as an essential resource, contributes to the growth of ecosystems and needs to be preserved and protected from potential harm. Persistent organic micropollutants, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and polycyclic aromatic hydrocarbons (PAHs), may cause harm due to their toxicity even in low concentrations. Monitoring these compounds in surface waters is crucial to preserve biodiversity, achieve good water quality, and investigate contaminated rivers to identify pollution sources.

To this end, the use of passive samplers to measure contaminant concentrations is becoming more widespread. Passive samplers offer high sensitivity, lack confounding matrix effects, and have a relatively easy deployment system. These samplers are usually extracted with Soxhlets, followed by purification of the extracts on chromatographic columns and quantification by gas chromatography-tandem mass spectrometry (GC-MS/MS). Currently, one of the biggest limitations of passive sampling for hydrophobic contaminants is the time- and resource-consuming analysis.

The aim of this work is to improve the efficiency of the current methodology, allowing passive sampling to become more routinely used for the monitoring of hydrophobic contaminants. An automated method, accelerated solvent extraction (ASE), has been tested and optimized to reduce the time and resources needed. For this purpose, samplers were spiked with target compounds under controlled conditions in the laboratory. In total, nine ASE methods were tested, and the results were compared with those obtained from Soxhlet extractions. The most efficient ASE method was chosen and will be tested on field samplers to assess the influence of environmental factors on the extraction. However, some PAH contaminations were detected. Thus, further optimization needs to be tested to avoid cross-contamination between samples, for example, by inserting more blank extractions between samples.

Improving the extraction of these passive samplers would facilitate their implementation on a larger scale for monitoring campaigns of micropollutants.

### **3.19.P-We199 Illicit drug epidemiology in Luxembourg using passive sampler based load balancing in wastewater treatment plants with different socio-economic population**

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Establishing load profiles of illicit drugs for urban communities at the inlets of wastewater treatment plants is challenging in terms of temporal representativeness. The problem is addressed by operating relatively cumbersome flow-proportional monitoring with autosamplers over several days. Besides being logistically limiting in terms of numbers of plants that can be monitored, the stability of the compounds during the storage time in the samplers is one of the issues adding to uncertainty. Here we used our formerly introduced weekly sampling with passive samplers to calculate inlet loads for illicit drugs. The approach calibrates the passive samplers at the low concentration variability of the outlet and calculates the inlet load via the elimination. The latter is obtained by the ratio of inlet/outlet mass of a compound in the passive samplers following a normalization with an inert compound to compensate for sampling rate differences. The approach was successfully applied for pharmaceuticals, biocides and household chemicals and was now tested on illicit drugs. For this purpose 9 wastewater treatment plants serving communes with socio-economic differences were monitored for 4 individual weeks during 2 seasons. The socio-economic gradient was established with respect to median salary of the connected communes and the age-structure of the population. Passive samplers were placed at the in- and outlets of the chosen plants and grab samples were taken at each switch/collection of the passive samplers to allow for sampling rate determination. Many of the illicit drugs were completely eliminated during wastewater treatment, jeopardizing the calibration strategy of using the outflow as a reference. Instead, using a mix of pharmaceuticals and doable illicit drugs, sampling rates were determined within the range of  $\log D$  at pH7 of the investigated compounds and delineated for the inlet. An uncertainty analysis revealed that the highest uncertainty did not reside in the monitoring or the analysis of the illicit drugs but the determination of the population equivalents during the monitoring periods. Median salary did only show a slight negative correlation with heroin but the age-group of young professionals (age 25-40) was correlated with the use of cannabis and cocaine (as measured by the metabolite benzoylecgonine)



### **3.19.P-We200 Prominent Features In Chemical, Physical And Ecotoxicological Analysis Of Seawater In The Genoa Harbor (Italy) By Means Of Passive Sampling Techniques**

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Assessing the current state of the global ecosystem is essential to fully understand the situation of the marine environment. Passive Sampling (PS) techniques are increasingly used for the integrated assessment of time-weighted average concentrations of both inorganic and organic compounds in a range of matrices, including water.

In the framework of Italian PNRR project "Return", the goal of this work is to monitor seawaters in Genoa harbour (NW Mediterranean Sea). PS will be exposed beginning in December 2023 with samplings being conducted once a season.

The so-called FL (Eastern entrance to the harbor near shipyards and mouth of the Bisagno stream), FB (inside the harbor in front of the Oil Terminal) and FP (Western entrance in front of the mouth of the Polcevera stream and sewage drain) are the three considered sampling sites. A wide range of anthropogenic chemicals may be present in seawater due to all the waterways connected to industrial and harbor activity and city and sewage discharges.

Organic chemicals will be sampled by Semi-Permeable Membrane Device (SPMD) and Polar Organic Chemical Integrative Sampler (POCIS) for lipophilic and hydrophilic compounds, respectively, and will be deployed for 21 days. Besides, Diffusive Gradients in Thin films (DGT) will be deployed for 7 days for metals and mercury sampling. When combined with the detection through high sensitivity and selectivity instrumentation at our disposal, such as GC-MS, LC-MS/MS, Hg-Analyzer and ICP-MS, this will enable the thorough assessment of the system studied.

On passive sampler extracts (polar, non-polar, metals) a battery of three bioassays with organisms belonging to different trophic levels (*Aliivibrio fischeri*, *Artemia salina*, *Dunaliella tertiolecta*) will be applied for the detection of ecotoxicological effects at the investigated locations.

Alongside the PS, sediment and water sampling will be also conducted at the end of the PS deployment. The detection of pollution at concentrations lower than ng/L will be possible thanks to the synergy of active and passive sampling systems. Physical measurements will be combined with water and sediment samplings, specifically temperature, conductivity, pH and oxygen, using a multiparametric probe (CTD).

The preliminary results of the first deployment will be presented. The chemical, physical and ecotoxicological characterization will enable a first assessment of the ecological risk due to the potential contamination of the water ecosystem.

### **3.19.P-We201 Passive sampling - results and comparison to national biota monitoring (within the WFD)**

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Under the European Water Framework Directive (WFD; 2000/06/EC), good chemical status must be achieved by 2027. To this end, European standards are set for priority substances, as laid down in the Priority Substances Directive (2013/39/EU). For eleven of these substances or groups of substances, which are poorly measurable in water, standards are also set in biota. Therefore Rijkswaterstaat (RWS), responsible for the main watersystem, has had a biota monitoring network operational since 2017.

If one or more biota EQS's are exceeded at a location, it should be considered whether or not upstream water bodies should also be monitored. For ethical, logistical and financial reasons, it is not desirable to monitor biota at all upstream locations. Moreover, many of the substances are ubiquitous and there is little scope for action if the substance is present at all sites. It may then not make sense to extend biota monitoring to upstream sites. Passive sampling (PS) could play a role in determining where other biota sampling should best take place.

In an earlier study RWS looked at the relative contents in the material used in the passive sampling (silicon sheets and Speedisk). A comparison of the calculated concentrations from the passive samplers and the concentrations measured in biota has now also taken place. During SETAC 2024 both kind of results will be presented.

During SETAC 2024 recommendations for the incorporation of passive sampling within future monitoring of RWS will also be presented.

### **3.19.P-We202 Calibration and field deployment of Ceramic Passive Samplers for monitoring of PFAS in groundwater**

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Groundwater contamination by per- and polyfluoroalkyl substances (PFAS) poses significant risks to human health and the environment. This study introduces Ceramic Passive Samplers (CPS) as an innovative and cost-effective strategy for monitoring PFAS in groundwater. The calibration process involved testing different sorbents, optimizing deployment times, and evaluating extraction conditions. CPS were calibrated over 30 days, demonstrating a linear calibration for all PFAS with sampling rates ranging from 1-10 mL/d. Stability assessments confirmed PFAS stability in water, crucial for CPS uptake. Deployments in highly polluted river basins, Besòs (Spain) and Kifissos (Greece), revealed CPS efficacy in detecting PFAS, with PFOA and PFOS as primary contaminants.

Twenty-five PFAS were analyzed, with CPS calibrated for the seven main PFAS. Oasis and Porapak sorbents showed optimal affinity and recoveries during calibration. CPS, deployed in boreholes for 30 days, exhibited no fouling, indicating sustained PFAS uptake. PFAS concentrations were calculated based on mass accumulation during deployment, revealing good agreement between CPS and grab sampling. The study validates CPS as a reliable method for PFAS monitoring, offering time-integrated concentrations without clogging issues and high analytical sensitivity.

### **3.19.P-We203 Passive sampler derived profiles and mass flows of Perfluorinated Alkyl Substances (PFAS) across the Fram Strait in the North Atlantic**

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Per- and polyfluorinated alkyl substances (PFAS) are a family of pollutants of high concern due to their ubiquity and negative human health impacts. The long-range marine transport of PFAS was observed during year-long deployments of passive tube samplers in the Fram Strait across three depth transects. Time weighted average concentrations ranged from 2.4-360 pg L<sup>-1</sup>, and 10 different PFAS were regularly observed. PFAS profiles and concentrations were generally similar to those previously characterized for polycyclic aromatic hydrocarbons (PAHs) at these sites. The detection of several anionic PFAS in “old” water demonstrated that they are not perfect water mass tracers, but are also transported to depth via settling particles. Mass flows of PFAS through the Fram Strait in and out of the Arctic Ocean were basically similar (97 29 Mg year<sup>-1</sup> northward flow, 107 39 Mg year<sup>-1</sup> southward flow). For FOSA, export from the Arctic Ocean via the Fram Strait exceeded import by Atlantic Water, likely due to preferential transport and deposition in the Arctic Ocean. These observations suggest that PFAS in the Arctic are governed by the same feedback loop previously described for PAHs in the region – with additional atmospheric transport delivering volatile PFASs to the Arctic, which then get exported via water masses.

### **3.19.P-We204 Atmospheric Passive Sampling Technique for Gas-Phase Perfluoroalkyl Carboxylic Acids**

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Perfluoroalkyl carboxylic acids (PFCAs), a sub-class of PFAS, are toxic compounds found ubiquitously in the environment. Studies have shown that atmospheric transport of PFCAs is an important transport mechanism which has sparked an interest in atmospheric measurements. Despite this, measuring mixing ratios of gaseous (g) PFCAs in the atmosphere has been an analytical challenge due to artifacts and biases, high maintenance, location limitations, and cost. Calibrated passive air samplers (PAS) alleviate these issues by offering a cheap, power-free, and low maintenance alternative. Atmospheric acids (e.g., nitric acid) have previously been measured by nylon PAS, which is selective for acids through chemisorption. Custom-built PFCAs (g) passive diffusion-based samplers employing nylon collection filters and polypropylene protective filters were comprehensively calibrated using a 1 m<sup>3</sup> chamber and were then deployed for measurements in the field. The PAS were deployed in several field locations: two remote locations (Saturna Island, British Columbia and Tadoussac, Quebec), as well as indoor and outdoor sites in Toronto, Ontario. Mixing ratios from PAS in outdoor urban air in Toronto were compared to orthogonal measurements to validate this new sampling method. The samples were quantified offline using ion chromatography with conductivity detection (IC-CD) or mass spectrometry (IC-MS) or gas chromatography mass spectrometry (GC-MS) via a novel derivatization technique. Our custom-built PAS were able to measure mixing ratios of TFA as low as 0.1 pptv over a 2-month period, demonstrating their ultra-trace measuring capabilities.

### **3.19.P-We205 Investigating the Uptake of Gaseous Aromatic Amines to Textiles**

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Aromatic amines (AAs) are mutagenic and/or carcinogenic compounds with environmental and industrial importance. AAs are found in indoor environments, originating from sources such as human activities like cooking, smoking, and hair dyeing. The risks associated with AAs in indoor spaces are amplified due to their often-elevated concentrations and the substantial amount of time people spend indoors. Furthermore, the impact of indoor contaminants extends beyond human exposure, as AAs indoor contaminants can be introduced into aquatic environments through cleaning and laundering of textiles. Indoor contaminants

can both deposit and sorb onto indoor textile surfaces such as carpets, curtains, and clothing. When textiles are laundered, sorbed chemicals can be released to wastewater and potentially affect receiving water bodies.

Recognizing the role of textiles as inadvertent indoor passive samplers (PS), we characterized the uptake of AAs from the gaseous phase of indoor air by cotton, wool, and polyester textiles under controlled conditions. A solvent extraction method for AAs in textiles was developed, relying on methyl tert-butyl ether, which demonstrated a higher extraction efficiency for AAs compared to acetonitrile and acetone. AA uptake to textiles was investigated using a series of chamber experiments to quantify textile-air partitioning. Textiles were uniformly spiked with a mix of AAs and placed in a glass chamber to equilibrate with the air inside. The chamber design allowed for the controlled extraction of a known volume of air by a vacuum pump. The analytes present in the air were sampled on a Tenax sorbent tube, and textile-air partitioning coefficient was calculated based on the concentrations in both the textile and the air. Textile-air partitioning coefficients for AAs showed a positive correlation with the octanol-air partition coefficient.

### **3.20.A Pharmaceuticals in the Environment – Risk Assessment, Regulation, and New Insights Into the Science Globally**

#### **3.20.A.T-01 Global Megatrends: the critical role of pharmaceuticals in health and environment**

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The role of pharmaceuticals in human and environmental health are critical to a sustainable future. Indeed health is a human right and access to medicines is key to the ‘*right to the highest attainable standard of health*’. But pharmaceutical residues are present in the environment and there is potential for harm to wildlife. We also know healthcare has a significant carbon footprint, whilst poor public health likely has an even bigger footprint. Therefore, healthcare’s impact on climate, biodiversity and pollution becomes a very complex problem; yet is critical to address the Megatrends and sustainability challenges. How do we secure the right to health while minimising the environmental burden arising from all aspects of healthcare?

There is growing ambition to reduce the carbon costs of medicines. But exclusively carbon-focussed approaches ignore other potential risks. For example; the potential for ecotoxicity, or environmental presence of residues after patient excretion are not addressed within strategies merely to reduce the carbon emissions. If we were to have to prioritise, which should we choose, a low carbon but high environmental presence or a higher carbon footprint but lower environmental presence medicine? And who should make that choice, a medic, an environmental or life cycle scientist, or a procurement specialist? Does selecting one medicine over another (where the potential risk of either may be characterised with a high degree of uncertainty) increase potential for regrettable substitution? Is this direction of travel proposed compatible with the patients right to health?

In the near future, if legislation changes, the right to the highest available standard of health for European patients may well be different to that of other parts of the world. Is the SETAC community equipped for this critical role that our life cycle assessment and fate & effect scientists will need to play? Are we training enough people? Are we targeting research in the areas to help clarify these societal concerns? The social and economic challenges of the Megatrends aim to ensure a healthy (European) population as the basis of a sustainable future. How we manage the environmental risk assessment of pharmaceuticals and the wider sustainability of medicines is critical to managing these Megatrend challenges ahead, and particularly important when ‘*putting health at the centre of climate change action*’.

#### **3.20.A.T-02 Pharmaceutical Contamination of European River Systems**

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While numerous studies have assessed the occurrence of different active pharmaceutical ingredients in surface waters in different regions of the world, these are often non-comparable due to differences in the analytical methods used. To address this challenge, a recent study applied a standardised approach to monitor the occurrence of a range of APIs in rivers from 104 countries (Wilkinson et al., 2022). While the study provided important insights into spatial variations in API exposure, as only one sampling timepoint was used, it provided no information on how concentrations varied temporally. This large-scale study was therefore performed to explore the spatial and temporal variability of concentrations of 56 APIs in river systems across Europe. The 56 target APIs were selected based on detection frequencies in the previous global monitoring study (Wilkinson et al., 2022) or had been prioritised by the Prioritisation and Risk Evaluation of Medicines in the Environment (PREMIER) project. A total of 281 sampling sites, spread across 31 European countries, were selected to cover a broad range of river types in terms of river size, river location and land use in order to capture spatial variation across European landscapes. A total of 2356 samples (including 172 field blank samples) were then collected at four timepoints (Summer, Autumn, Winter and Spring, 2022-2023) across a 12 month period. Based on preliminary findings for the four countries Albania, Austria, Belgium, and Cyprus, Belgium exhibits the highest level of pollution with seasonal average cumulative concentrations of 5.4 µg/L across all sites, whereas Austria holds the lowest level (1.0 µg/L). Temporal variations in API concentrations and the number of API detections varied between countries. This result could potentially be attributed to a combination of factors, including population density, pharmaceutical usage, chemical properties (hydrophobicity, degradation rate), WWTP removal efficiency and hydrology (flow rate, DOC).

### 3.20.A.T-03 Temporal Analysis of EcoPharmacoVigilance Data; Insights from Measured Environmental Concentrations of Active Pharmaceutical Ingredients (APIs) since 1996

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Advances in the sensitivity and throughput of monitoring analytics and the growing interest in PiE (Pharmaceuticals in the Environment) are continuing to deliver an exponential increase in MECs (measured environmental concentrations) gathered and reported in the scientific literature. The EcoPharmacoVigilance (EPV) process implemented at AstraZeneca has identified over 46,000 aquatic MECs dating back to 1996 that are made publicly accessible via an online dashboard. Temporal analysis of this data allows insights into global and regional trends of reporting and environmental risk over more than two decades.

The EPV process implemented at AstraZeneca was published in 2013. Under this process, MECs reported for our portfolio (and some legacy) small molecule APIs were extracted from the published, peer-reviewed literature. Of the totality of aquatic MECs, data reported for *surface water* (30,530 MECs) were subjected to further analysis. This analysis shows that amounts of reported water monitoring data has been rising steeply over the past two decades, first in Europe followed by the US (since 2008) and other global regions. The vast majority, 99.3% of *surface water* MECs reported to date (Q2 2023) indicate a low or insignificant risk (risk quotients derived using PNECs from published ERAs and grouped by risk according to Fass guidance).

Where AstraZeneca APIs were tested for, quantifiable concentrations were typically detected on half of all occasions. Mean, median and 95<sup>th</sup> %ile risk quotients calculated for discrete 3-year reporting periods are well below 1 when taking the full data set into account. When excluding non-detects, the corresponding conservative risk quotients of quantified concentrations are also below 1, with two exceptions; the 95<sup>th</sup> %ile RQ of 1.18 for 2002 to 2004 and the mean RQ of 1.26 for 2020 to 2022.

Based on this preliminary analysis of temporal trends, the available data does not indicate a global increase in surface water risk.

Further analysis of this data (and any additional data reported during year 2023) will investigate;

- Temporal trends at finer (year by year) resolution, for specific (insignificant, low, moderate, high) risk groups and for discrete global regions (e.g. Europe, USA, Asia)
- Focused analysis of specific, data rich APIs.

This will provide insights into global regions of heightened concern and impacts of the quality of data reporting on the utility of existing monitoring data for risk assessment and management.

### 3.20.A.T-04 DISTRIBUTION, BIOACCUMULATION AND ECOLOGICAL RISK ASSESSMENT OF CONTAMINANTS OF EMERGING CONCERN IN THE FRESHWATER-SEDIMENT-FISH SYSTEM

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Contaminants of emerging concern (CECs), including pesticides and pharmaceutically active compounds (PhACs), cover a diversity of substances with broad physico-chemical properties. Their extensive use has resulted in their presence in diverse aquatic ecosystems, impacting water quality. Some of these compounds have the ability to accumulate in the tissues/organs of aquatic organisms, generating adverse effects on their endocrine or reproductive system. The discharge of effluents from wastewater treatment plants (WWTP) has been identified as a significant source of PhACs contamination, with concentrations that can reach effect thresholds, indicating possible adverse ecological effects. This study evaluated the ecological risk and bioaccumulation potential of 32 CECs in a Spanish river, particularly in organs/tissues of common carp (plasma, muscle, skin, liver, kidney, brain, and heart). In addition, environmental concentrations in freshwater and sediment were assessed at five sampling sites upstream and downstream of the WWTP. The highest total concentrations ( $\Sigma$ CECs) for water and sediments were six and eighteen times higher for the sampling sites downstream of the WWTP (R4: 12.4  $\mu\text{g L}^{-1}$  and R3: 30.1  $\mu\text{g L}^{-1}$ ) compared to those upstream (R1: 2.08  $\mu\text{g L}^{-1}$  and R2: 1.66  $\mu\text{g L}^{-1}$ ). Half of the contaminants were detected in fish tissues and organs (5M1H-benzotriazole, amantadine, benzotriazole, bisoprolol, BPA, BPS, BPF, caffeine, cotinine, diazepam, hydrochlorothiazide, metoprolol, desmethylvenlafaxine, sotalol, terbutryn, and venlafaxine), and the highest mean concentrations occurred for amantadine in kidney (158  $\text{ng g}^{-1}$  w. w), and liver (93  $\text{ng g}^{-1}$  w.w) and for terbutryn in brain (50  $\text{ng g}^{-1}$  w.w). Experimental bioaccumulation factors (BCFs) indicated substantial bioaccumulation potential (greater than 500) for amantadine, diazepam, and terbutryn in the kidney, liver, and brain. Individual risk quotients (RQs) suggested that 63% of the contaminants had a low risk for common carp, but caffeine and terbutryn showed elevated RQs for fish (RQ<sub>max</sub> 11 for caffeine and 10 for terbutryn). The mixed risk quotient (MRQ) revealed medium risk for most carp (between 2 and 14) and

elevated risk for downstream sites (between 2 and 30). These results underscore the need for comprehensive risk assessments that span multiple environmental compartments to better to understand the actual exposure levels of an aquatic ecosystem.

### **3.20.A.T-05 Engaging Citizen Scientists to Support Monitoring of Contaminants of Emerging Concern in Coastal Environments**

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Contaminants of emerging concern (CECs) are detected in the environment as a result of widespread anthropogenic activity. With wastewater discharges serving as one common entry route of pharmaceuticals into water bodies. Therefore, it is important to understand the influence of wastewater treatment plant (WWTPs) discharges and combined sewer overflow (CSO) events on the presence of CECs and is herein analysed for a coastal environment in the UK with input from 5 different WWTPs. Citizen scientists collected samples from 22 different sites within the Chichester and Langstone harbours. Two primary and two minor sampling campaigns were conducted aimed to capture seasonal variations and evaluate potential CSO event impacts during periods of drought and heavy rainfall. In addition, biota samples from 6 species representing important ecological groupings were collected and analysed to better understand the potential for bioaccumulation. A targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) method was employed to analyse 149 compounds covering pharmaceuticals, pesticides, and recreational drugs. An additional 341 analytes were screened to extend the suite of chemicals monitored. The analysis revealed the occurrence of over 150 compounds and patterns in CEC concentrations with influences from WWTP discharges and CSO events. During dry weather conditions, concentrations of specific pharmaceuticals exhibited a consistent profile in the low ng L<sup>-1</sup> range, indicating a continuous input from anthropogenic sources. An increase in concentrations was detected during wet weather conditions for selected sites. Moreover, CSO events were identified and captured as drivers of short-term spikes in CEC concentrations at sites close to these discharge points. Notably, some compounds that were previously not detectable at these sites emerged temporarily after CSO events. For other compounds previously detected, concentrations increased up to hundred-fold, with some concentrations reaching up into µg L<sup>-1</sup> range. This study provides an insight into the presence of CECs and the findings emphasize the significance of considering both WWTP discharges and CSO events in assessing the environmental fate of pharmaceuticals and other CECs in coastal environments and highlights the collaborative integration of local citizen scientists that can support regional mitigation efforts.

### **3.20.B Pharmaceuticals in the Environment – Risk Assessment, Regulation, and New Insights Into the Science Globally**

#### **3.20.B.T-01 Understanding the Variability of Poultry Litter on the Degradation Rates of Veterinary Medicines and Feed Additives.**

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The potential environmental impact of veterinary medicines and feed additives is assessed through an environmental risk assessment as part of the regulatory process. Simulating manure degradation can be used as an approach to refine environmental exposure and currently the guidance provided by the European Medicines Agency in 2016 only requires testing in one source of manure per animal type. Most of the research exploring degradation of veterinary medicines has been conducted on pig slurry or cattle manure, leading to an specific to these 'liquid' manures. In comparison, poultry litter is relatively understudied. The variability of poultry litter properties and the potential effect of this variability on the rate of degradation of veterinary medicines is relatively unknown. Subsequently, the current guidance on the number and type of litters to be used may not accurately evaluate the true environmental risk posed by these compounds. A series of experiments have been designed to explore the impact of poultry litter collected from different farms on the degradation of five veterinary antibiotics: tiamulin, trimethoprim, sulfadiazine, monensin and salinomycin. Three litter types that differed in the type of chickens reared and the type of bedding material were collected from three separate farms and used in simulated degradation studies performed according to the harmonised experimental guidance published by the European Medicines Agency in 2011. Results revealed that the rates of degradation for salinomycin differed significantly ( $p = 2.0 \times 10^{-16}$ ) between the poultry litters, in the litter collected from one farm, salinomycin was persistent (DT50 >1000 days) and in the litters from other farms it degraded rapidly (DT50 <1). Our results suggest that we need to better understand how the variability in the chemical and biological properties of poultry litters can influence degradation processes. Based on these initial findings, a number of litters from different farms may be needed to perform manure degradation studies to deliver a comprehensive, and realistic environmental risk assessment.

#### **3.20.B.T-02 Acidification increases the toxicity of diclofenac to aquatic organisms**

*Katarzyna Bethke, Klaudia Kwidzińska, Weronika Koczur, Anna Bialk-Bielińska and Magda Caban, Department of Environmental Analysis, University of Gdansk, Poland*

Pharmaceuticals and their metabolites are among the challenges of modern ecotoxicology and environmental chemistry. Even at low concentration levels, they can be dangerous to non-target aquatic organisms. It turns out that the toxicity of pharmaceuticals can be affected by global warming effects, such as an increase in average temperature and a decrease in pH in water bodies. The direction of toxicity change with acidification is problematic to predict due to diverse chemical structure and mode of action of pharmaceuticals.

We therefore decided to investigate the effect of acidification of water bodies on changing the toxicity of diclofenac, a drug from the non-steroidal anti-inflammatory group. Toxicological tests were conducted against two model organisms: *Daphnia magna* and *Lemna minor*. Acute and chronic tests were carried out in accordance with OECD guidelines 202, 221 and 211. These tests allowed, among other things, the determination of EC<sub>50</sub> parameters, thus confirming the negative effect of lowering the pH on the toxicity of diclofenac to the test organisms (mortality, inhibition of growth or reproduction). Interestingly, quantitative analysis of diclofenac (HPLC - DAD) during tests with *L. minor* confirmed the degradation of the pharmaceutical during the test, which was not observed during tests with *D. magna*. The enhanced toxicity to the duckweed may therefore be due to exposure to any of the diclofenac derivatives. It is possible that changing the pH of water bodies also alters the toxicity of pharmaceutical degradation products. To date, there has been little work on the effects of changing the toxicity of pharmaceuticals on aquatic plants, so this study adds to the body of knowledge on the subject and confirms the need for further experiments on the effects of climate change on the toxicity of pharmaceuticals and their derivatives.

### **3.20.B.T-03 Fish Show Altered Reproductive Behaviours in the Wild Following a Whole-Lake Exposure to an Estrogenic Pharmaceutical**

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Reproductive behaviour has become a key endpoint in studies assessing the impacts and risks of endocrine disrupting chemicals and pharmaceuticals to wild fish populations. Yet, it is exceedingly difficult to test whether widely accepted behavioural responses observed in the laboratory are also manifested in the same way under natural conditions in the field, and what the repercussions of this might be for exposed populations. To close this gap, we measured the reproductive behaviour of male fathead minnows (*Pimephales promelas*) from video recordings taken over multiple years before, during and after the synthetic estrogen ethinylestradiol (EE2; used in birth control pills and hormone therapies) was added to a lake in a whole-lake exposure study. In line with previous laboratory EE2-exposure studies, we found that males from the EE2-exposed lake reduced the amount of time they spent tending to their nest when compared to fish from reference lakes. However, in contrast to prior laboratory studies, we found that males from the EE2-exposed lake showed increased nest defence as measured by aggression towards conspecifics. We also found that social dynamics at the nest changed in the EE2-exposed lake, where more non-reproductive conspecifics were present during nesting and interrupted the focal males. We evaluated the expression of male secondary sex characteristics alongside behaviour and found that males from the EE2-exposed lake showed marked reductions in secondary sexual characteristics compared to males from reference lakes in the final years of exposure. We will connect our new behavioural findings to already established physiological and population-level effects measured during this whole-lake exposure study. Our findings contradict the generally held view of reduced spawning aggression by estrogen-exposed males, and we highlight that the complex ecological and behavioural interactions that occur in natural systems provide a challenging but necessary arena in which to pursue ecotoxicological studies.

### **3.20.B.T-04 Antibiotic and Emerging Antibiotic Resistance Assessment in the Ebro Delta and the Albufera of Valencia reservoirs (Spain)**

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Urban rivers are impacted ecosystems which may play an important role as reservoirs for antibiotic-resistant (AR) bacteria. The primary goal of this work was to assess antibiotics, the prevalence of antibiotic resistance through the study of antibiotic resistance genes (ARGs) and bacteria (ARBs), in two protected reservoir areas in Spain, the Ebro Delta (NE of Spain), which is the biggest river in the north of Spain, and the Albufera of Valencia, a freshwater lagoon on the Gulf of Valencia (E of Spain).

Main antibiotics groups were assessed by ultra-performance liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS). ARGs were detected using qPCR to assess aquatic habitats' emerging microbial contaminants vulnerability and ARBs like *Escherichia coli* were grown by cultivation methods.

The main results of suspect screening showed the presence of  $\beta$ -lactams, tetracyclines, macrolides, sulfonamides, quinolones and DHFR inhibitors. The further analysis for the unequivocal identification confirm the presence of quinolones such as ciprofloxacin and ofloxacin, the macrolides such as clarithromycin, tetracyclines such as tetracycline, and sulfonamides including sulfamethoxazole, and trimethoprim. Antibiotics were detected in almost all the samples with some seasonal variations, where the concentrations were ranging between the MLOQ and 104.9  $\mu\text{g/L}$ . In the case of bacteria, despite the microbial load decreases in effluent compared to influent wastewater (2-3 log<sub>10</sub> on average), the reductions between upstream and downstream wastewater do not comply with the latest European standards, Regulation (EU) 2020/741 of the European Parliament and of the Council on minimum requirements for water reuse.

### **3.20.B.T-05 Refining the Predicted Environmental Concentration (PEC) of human pharmaceuticals; effect of region-specific dilution factors and wastewater volumes**

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To classify environmental risk of a human pharmaceutical when completing an environmental risk assessment, the PEC resulting from patient use is compared to the Predicted No Effect Concentration. There are various methods of calculating the PEC which require the assessor to estimate the environmental concentration using the quantity of Active Pharmaceutical Ingredient (API) intended for patient use. However current methods use default values for wastewater production ( $V_{ww}$ ) and dilution factor of wastewater by surface water flow (DF) regardless of where the pharmaceutical product is sold and ultimately excreted. The European Medicines Agency (EMA) default values are from technical guidance dated 2003, yet in the last 10 years at least two papers have been published estimating region specific values for both DF and  $V_{ww}$ . The purpose of this study is to apply these region-specific values when calculating the PEC, to assess the impact of using default versus refined values, and how this may affect the environmental risk classification of an API.

2022 sales data of 41 AstraZeneca APIs were assessed, and the highest per capita sales were identified for each. These data were used to calculate the PEC as per the equation in the Fass guidance for the four combinations of default or refined DF and  $V_{ww}$  values. 36 APIs reported a reduction in PEC when refined with both region-specific values, resulting in 4 APIs being assigned to a lower risk category. 5 APIs reported an increased PEC when fully refined, but none changed risk category. PEC values were more sensitive to the DF than  $V_{ww}$  parameter, resulting in an average difference to the default PEC of 18%. Regional analysis was conducted for one API, which showed that when refining both DF and  $V_{ww}$ , Saudi Arabia reported high environmental risk, despite per capita use 2 orders of magnitude below that of the country with the highest per capita sales. The PEC derived using default EMA values is generally more conservative than regional specific PECs and full refinement results in a decrease in PEC for more than 80% of APIs assessed. It was also highlighted that refinement using region-specific DF and  $V_{ww}$  values must be combined with country-specific sales, as regions of highest per capita sales may not always be where an API poses the highest environmental risk. This offers potential risk management opportunities, as it would allow assessors to prioritise and target such efforts based on locally predicted risk.

### **3.20.P Pharmaceuticals in the Environment – Risk Assessment, Regulation, and New Insights Into the Science Globally**

#### **3.20.P-Th344 Metabolites Are Overlooked in Environmental Risk Assessment and Monitoring of Pharmaceuticals: The Case Study of Pantoprazole**

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After application, pharmaceuticals and their metabolites are excreted via urine and feces. Since many compounds cannot be completely eliminated by conventional wastewater treatment plants (WWTPs), they are regularly detected in receiving waters. To date, approaches to environmental monitoring as well as risk assessments for regulatory purposes focus on the parent compounds of pharmaceuticals. For instance, the environmental risk assessment of medical products for human use in the EU largely ignores transformation in regulatory authorization procedures, allowing for a so-called total residue approach in which the environmental fate and toxicity of metabolites are assumed to be covered by that of the original pharmaceutical.

Pantoprazole (PPZ) is a top-selling PPI worldwide. Despite the large consumption volumes, concentrations of PPZ in urban waters are comparatively low, which can be explained by the extensive metabolization of PPZ in the human body. By screening urine from a PPZ consumer and WWTP effluent for the presence of known PPZ metabolites by high resolution mass spectrometry, 4'-O-demethyl-PPZ-sulfide (M1) was identified as the most promising PPZ related compound for environmental analysis. M1, which, to the best of our knowledge, has not been described yet as a human metabolite of PPZ, was found to be ubiquitously present in WWTP influents and effluents (up to 3 µg/L) as well as in wastewater-impacted surface waters (up to 1.4 µg/L) in Germany. On average, the concentrations of M1 were about 30 times higher than those of the parent compound PPZ in the analyzed surface water samples. Concentrations of M1 in suspended particulate matter from the Rhine River at Koblenz showed a strong increase between 2005 and 2015 and were highly positively correlated with the annual prescription volumes of PPZ in Germany. Laboratory scale adsorption experiments demonstrated that post-treatment with activated carbon can substantially improve the removal of M1 during wastewater treatment. Laboratory ozonation experiments revealed fast removal of M1 but also the formation of several transformation products during the ozonation of M1. The presented study shows that information on the absorption, distribution, metabolism, and excretion of medicinal products after administration must be taken into account in comprehensive monitoring programs in order to adequately describe their environmental occurrence and relevance.

#### **3.20.P-Th345 A Refined Read-Across Approach to Support Environmental Assessment of Data-Poor Pharmaceuticals**

**Tim Verslycke, Ifeoluwa Bamgbose and Janet Vo, Gradient**

Environmental assessments are required for market approval of new active pharmaceutical ingredients (APIs) in the US and the EU. However, ecotoxicological information to support such assessments is often lacking, particularly for drugs that were approved prior to the implementation of environmental assessment regulations and for new drugs that qualify for a categorical exclusion. Evaluating environmental risks of drugs lacking ecotoxicity data typically requires the use of quantitative structure-activity modeling (QSAR) or read-across approaches. QSAR ecotoxicity modeling can be unreliable for APIs and requires expert application and interpretation. Therefore, in 2012, we proposed a simple read-across approach based on drug therapeutic class and an experimental aquatic toxicity dataset containing 184 APIs belonging to 14 therapeutic classes

(Verslycke *et al.*, 2012; SETAC Europe Annual Meeting, Berlin, Germany). In 2023, we updated our 2012 therapeutic class read-across approach using an expanded experimental aquatic toxicity dataset containing 314 APIs (Bamgbose *et al.*, 2023; SETAC North America Annual Meeting, Louisville, USA). Here, we propose a further refinement of our read-across approach by using subclasses that consider human therapeutic target and presence/absence of that target in ecological taxa (algae, invertebrates, or fish).

### **3.20.P-Th346 Exposure Assessment of Pharmaceuticals in the Northern Adriatic Sea: Emission Inventory and Environmental Modelling**

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Pharmaceuticals are fundamental to effectively treat a multitude of diseases both in humans and animals but, at the same time, there is evidence of risks to the environment and, particularly in relation to antimicrobial resistance, to human health caused by the environmental release of these chemicals during their manufacture, use and disposal. However, the monitoring of pharmaceuticals in the environment is still limited, especially for coastal waterbodies. The use of predictive exposure models has been identified as a complementary, cost-efficient tool to support the investigation of the behaviour and environmental fate of these contaminants and the assessment of associated risks.

The Northern Adriatic is a narrow and shallow sea, where the variety of temperature, salinity, and sedimentation patterns leads to the presence of different and unique habitats for marine species. This area is also subjected to several anthropogenic pressures, such as high nutrient loads from agricultural activities, discharges of urban/industrial wastewaters, and in general a diversity of anthropic activities (such as tourism, transport of freights and people, mining, aquaculture, etc.) leading to the release of various types of pollutants. Among these pressures, the discharge of active pharmaceutical compounds raises particular concern, especially due the lack of information regarding their concentration in water and sediments.

The aims of this work, developed within the iNEST project, are: (i) to develop an emission inventory of several active pharmaceutical ingredients in the study area, (ii) to use state-of-the-art modelling tools to investigate the environmental distribution and fate of these contaminants in the Northern Adriatic, with particular attention to the processes involved in their natural attenuation, and (iii) to define a risk-based priority list of contaminants specific to the case study area, taking into consideration the overall mass balance of the target pollutants.

The emission inventory was developed by integrating pharmaceutical sales data for human consumption obtained from each Regional authority within the Northern Adriatic drainage basin with river flow, census and geographical information through statistical data treatment and GIS-based spatial analysis. In addition, a preliminary modelling exercise was carried out by applying the newly-developed ChemicalDrift model, a chemical transport module part of the open-source Lagrangian framework OpenDrift.

### **3.20.P-Th347 Can the Enantiomeric Fraction of Chiral Pharmaceuticals be used to Identify Septic Tank Discharges?**

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Pharmaceuticals are emerging contaminants in the environment and they are found in various water sources worldwide in the ng to  $\mu\text{g L}^{-1}$  range. They are often chiral, existing as at least two enantiomers. Enantiomers are mirror images of each other with identical chemical structures but different spatial arrangements. Pairs of enantiomers (S-(+) and R-(-)) can demonstrate enantioselectivity in their environmental occurrence, fate, and toxicity. Consequently, enantioselective analysis is an important tool in risk assessment and source identification for chiral drugs.

Concentrations and enantioselective composition of 28 chiral pharmaceuticals and metabolites were determined in septic tank wastewater and river microcosms. Enantioselective separations were achieved using a UHPLC-MS/MS system and a ChiralPak IG-U and a InfinityLab Poroshell 120 Chiral-V column, respectively.

No significant difference was found between the enantiomeric composition of septic tank influent and effluent for chlorpheniramine, citalopram and naproxen, suggesting that no enantioselective degradation occurs. In line with its enantiopure dispensation, R-(-)-naproxen was either not detected in septic tank wastewater or found in substantially lower concentrations than S-(+)-naproxen. Enantioselective degradation of pharmaceuticals is typically observed in conventional wastewater treatment systems (WWTS). Concentration data shows no or limited removal of pharmaceuticals through septic tanks. Overall, the unchanged enantiomeric composition in septic tank wastewater and limited reduction of total concentrations, indicate that pharmaceuticals are removed to a lesser degree in septic tanks than in conventional WWTSs.

Pharmaceuticals are degraded in abiotic and biotic river microcosms in light conditions over 13 days. The degradation processes of chlorpheniramine and citalopram are not enantioselective. Inversion from S-(+)- to R-(-)-naproxen was not observed. Overall, the similarity between abiotic and biotic microcosms suggests that in river water, microbial degradation is negligible over other degradation processes, such as photodegradation.



Potentially, the unchanged enantiomeric composition in septic tank wastewater, can be used to distinguish between pharmaceutical discharges from septic tanks and conventional WWTSs in the environment.

### 3.20.P-Th348 Seasonal study of the presence of pharmaceuticals in A Coruña estuary (NW Spain)

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In last years an increase in the presence of potentially toxic compounds known as contaminants of emerging concern (CEC) was observed due to the population growth and industrial development. Among them the pharmaceutical compounds and their metabolites are highly spread. Their presence in the environment is related with two main sources, firstly wastes from laboratories, hospitals and agriculture, and secondly, their excretion after their consumption, which appears in wastewaters. Not all organic pollutants are removed at WWTPs, so some pharmaceuticals are being found in the water. The aim of this work is to study the presence and behavior of 56 pharmaceuticals using liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS (QqQ)) for their determination. The seasonal study was performed collecting samples from the A Coruña estuary (NW Spain) making a radial of 5 points during 2022, analyzing each point in every season. 16 pharmaceutical compounds including antibiotics, NSAIDs, cardiovascular drugs and psychiatric drugs among others were found in levels from 0.1-67.5 ng/L with different occurrence and behavior. Pharmaceuticals were found in all samples, 5 of them (Azithromycin, Carbamazepine, Clarithromycin, Diclofenac and Erythromycin) are proposed to be regulated by the EU. It appears that in summer and spring are the seasons with the higher concentration of analytes in the water. Antibiotics and NSAID were found in specific places while the rest of therapeutic classes seems to be more distributed.

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### 3.20.P-Th349 Evaluation of the Occurrence and Fate of Pharmaceuticals in North and South Mediterranean Intermittent River Basins

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Most river basins are subjected to several anthropogenic inputs, including wastewater treatment plant (WWTPs) discharges and urban/storm runoff waters, which collectively affect water quality. Despite the use of WWTPs to manage and treat wastewater, their effluent may still contain contaminants, including pharmaceuticals, as conventional WWTP do not fully eliminate them. This study analyzed the presence of pharmaceuticals in five countries: Spain, France, Italy, (North Mediterranean) and Algeria and Tunisia (South Mediterranean). In Tunisia and Algeria, WWTPs are sometimes over-exploited and industries often release wastewater directly into river basins. In addition, the lack of regulations regarding pharmaceutical concentration in surface water and the limited monitoring makes it interesting to investigate their presence and impact. Hence, different intermittent rivers from each site were sampled and possible differences between the North Mediterranean and the South Mediterranean basins were investigated. A total of 82, mainly pharmaceuticals and other organic contaminants, selected based on their occurrence and ubiquity in the aquatic environment were screened and quantified using high-resolution mass spectrometry (Q-Exactive-Orbitrap-MS). The presence and potential differences in contamination levels across the five countries were investigated. The studied river basins from France and Algeria reported lowest concentrations while the basin in Tunisia reported the highest concentrations. The most remarkable class were the industrial compounds where significant differences between Tunisia and the four other countries were observed. As for Italy, the highest concentrations belonged to the antihypertensive group. Regarding specific contaminants, caffeine concentrations were outstanding in Tunisia compared to the rest of countries, and Italy presented remarkable concentrations of irbesartan and valsartan acid, a sartan group metabolite. A prioritization list based on compounds with the highest frequency of predicted no-effect concentration (PNEC) exceedance was compiled and a risk assessment approach was performed. Tunisia was the country with the highest concentration for the majority of the prioritized compounds, emphasizing the need and requirement of attention for regulations and researchers for further monitoring efforts.

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### 3.20.P-Th350 Solid-phase extraction and chromatographic determination of diazepam, alprazolam and clonazepam in wastewater samples

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Pharmaceutical pollutants entering the aquatic environment have become a growing environmental concern. These pharmaceuticals are unique pollutants because of their special characteristics and behaviour that cannot be simulated with other organic pollutants. The untreated wastewater effluent that contains pharmaceuticals poses considerable threat to the aquatic ecosystem because of the negative effects of non-target organisms in the water. Recent years have seen a growing concern about the benzodiazepines, as emerging pollutants, and their effects on the aquatic environment. These compounds are nowadays widely detected in sewage wastewater. It is important to increase the emphasis on the characteristics of the benzodiazepines in order to differentiate them from industrial chemical compounds. In this study various solid phase extraction techniques have been employed focusing on isolation of benzodiazepines in wastewater matrices. Employing this methodology has shown improved detection and analysis of diazepam and clonazepam as benzodiazepines.

Preliminary results have shown the diazepam concentrations to range between 1 to 9 ppm in seasonal wastewater samples analysed, with higher concentrations during the winter season. In the case of clonazepam and alprazolam, concentrations ranged between 0.4 to 2 ppm. Seasonal characteristics of the benzodiazepines are discussed along the trends observed for the water quality characteristics of the final treated wastewater.

### **3.20.P-Th351 Anthropogenic Gadolinium And Correlations of Pharmaceuticals Along River Wutach (Black Forest Germany)**

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In the recent years more and more reports revealed, that pharmaceuticals and residues from medical treatments reach sewage treatment plants via wastewater. However, the processes in the sewage plants are not suitable to degrade or retain those compounds, thus detectable quantities of such compounds travel through the sewage plants, and are discharged into river water. From there they can reach by exchange processes groundwater and from sewage sludge applications the soil zone. Gadolinium (Gd) and pharmaceuticals against modern industrial society diseases (such as antiepileptic drugs, pain relievers, antibiotics as well as beta-blockers) and contrast agents (e.g. for x-ray and magnetic resonance imaging MRI) can usually be detected in traces in almost every river in densely populated regions with state of the art medical treatment facilities. The highest concentrations are usually antidiabetics, x-ray agents, antihypertensives (drops blood pressure) and diuretics, as well as Gd as contrast agent for MRI. In general, there are only a few studies on anthropogenic Gd accumulation and pharmaceuticals in natural catchment areas, almost no reports in Germany. Thus, in this study we investigated the occurrence and distribution of such pharmaceuticals and Gd in the relatively natural catchment of the river Wutach in the Southern Black Forest. We took 21 samples from various locations along the river. From the concentrations, correlations were checked and mass fluxes were calculated. First results show, that even in the natural Wutach Valley all target compounds could be detected in traces, only in the spring area with very low anthropogenic impact (few farm houses, no hospital etc.) no excess of Gd could be found and pharmaceuticals were below detection limit. With the occurrence of the hospital in the catchment, an anthropogenic excess of Gd in the river water can be calculated, even for locations up-stream of the hospital (excretion at home after application). Correlations found are probably "spurious correlations", but it can be concluded, that patients with diabetes may have high blood pressure and take pain reliever, patients with schizophrenia take antidepressive medicaments, combinations of pharmaceuticals are subscribed to control blood pressure (e.g. candesartan and hydrochlorothiazide). A weak correlation of candesartan and 4(5)-methylbenzotriazole (dishwasher ingredient) may point to the excretion of blood pressure control medicaments mostly at home.

### **3.20.P-Th352 Perils of Pet Pollution: Monitoring of Companion Animal Parasiticides in English Surface Waters**

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Parasiticides are commonly and extensively used to treat pets. With 21 million cats and dogs within the UK alone, there is growing concern over the potential impacts of these chemicals in the environment. In the UK, 31 active ingredients are used as parasiticide treatments in pets and, following use, these can enter the environment via down the drain emission from wastewater treatment plant (wwtp) effluent discharges and direct emissions from dogs swimming in the aquatic environment. However, only a limited number of studies have focused on determining the concentrations of pet parasiticide compounds in freshwater systems, with most focusing on a small number of compounds namely, fipronil and imidacloprid. Furthermore, data are lacking on concentrations within sediment in the aquatic environment. Levels of aquatic environmental exposure for a broad range of parasiticides due to their use within and on pet parasiticides are, therefore, largely unknown. This monitoring study was therefore performed to generate exposure data for companion animal parasiticides in English surface waters and to understand the relative importance of different pathways of release of these substances to the environment. Approximately 350 water and sediment samples will be collected across 23 sampling sites across 12 different locations across England. Seven sampling locations have been selected to capture the contribution of dogs swimming to parasiticide concentrations in the aquatic environment and five sampling locations have been selected to determine the influence of wwtp effluents on levels of exposure. The concentration of a broad range of compounds from a number of chemical classes including neonicotinoids, isoxazolines and macrocyclic lactones will be determined. The monitoring campaign is on-going. The current focus of this work is to undertake targeted analysis of the sediment and water samples to determine the concentration of pet parasiticide

compounds associated with down the drain and direct emission pathways to the aquatic environment. The study will generate knowledge on exposure of these understudied substances which will then help to support the assessment of the potential environmental risk posed by pet parasiticides.

### **3.20.P-Th353 Occurrence and Human Exposure Assessment of Pharmaceutically Active Compounds (PhACs) in Indoor Dust Collected from Spanish Homes, Schools and Offices**

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The increasing consumption of medicines could raise the presence of pharmaceutically active compounds (PhACs) in the environment and lead to health problems. Although PhACs occurrence in other environmental compartments has already been studied, little is known about their presence in indoor locations. The presence of any of these compounds in the indoor dust is of great concern since we spend most of our time in indoor environments, and this may be a source of human exposure to these compounds.

To address this issue, 85 indoor dust samples were collected in central Spain from 14 homes, 23 offices, and 48 schools, from kindergartens (n=21) and high schools (n=27), during 2022. Then, the presence of 23 analytes belonging to several groups of PhACs as antibiotics,  $\beta$ -blocker agents, psychiatric drugs, analgesics, anti-inflammatories, lipids regulators, fungicides and anthelmintics were determined. Indoor dust samples (1g) were spiked with deuterated internal standards and extracted by the QuEChERS method consisting of a mixture of Milli-Q water and acetonitrile acidified with 1% acetic acid in the presence of magnesium sulfate and sodium acetate. Instrumental analysis was carried out with a UHPLC-MS/MS system.

Chromatographic separation was made by an ExionLC system coupled to a Triple Quad™ 3500 MS/MS system for the MS/MS analysis. The method was validated according to SANTE/2020/12830 and SANTE/11312/2021 criteria and method limits of quantifications ranged between 10 and 50 ng/L. Recovery for deuterated standards were always between 70 and 120%.

Suspended and deposited dust was collected separately in schools and offices, but no statistically significant differences were found between both types of dust. Acetaminophen, clotrimazole, thiabendazole and the metabolite anhydroerythromycin were quantified in >50% of the samples with median concentrations of 166 ng/g, 75 ng/g, 25 ng/g and 2 ng/g, respectively. 13 PhACs were quantified in less than 9% of the samples with concentrations ranging between 10 ng/g and 630 ng/g. In contrast, 9 PhACs were below LODs in all cases. Concentration differences between locations were investigated, resulting in acetaminophen being the main PhAC quantified in most of the samples, especially in homes. Interestingly, the presence of other target analytes such as thiabendazole highlights possible non-pharmacological sources.

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### **3.20.P-Th354 Aquatic-to-Terrestrial Transfer of Pharmaceuticals: Insights from Riparian Spiders**

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The presence of pharmaceuticals in surface waters, and their effects on wildlife, has been widely reported, yet the risk of exposure for aquatic organisms and transference to terrestrial systems has been poorly characterized. Pharmaceuticals can be transported from aquatic systems to land when terrestrial predators such as riparian spiders, birds, or bats feed on emergent insects, or drink contaminated water. Riparian spiders can be effective sentinels to monitor aquatic to terrestrial contaminant flux because they are widespread, abundant, relatively sedentary, occupy high trophic levels, and are important prey for vertebrates. Here, we assessed the pharmaceutical load of riparian spiders from 26 sites across Sweden. The samples were collected as part of a community science project where high school students were trained to collect spiders from riparian zones and water samples from the closest water body. We screened both types of samples (totaling 565 spider samples and 220 water samples) for 95 pharmaceutical compounds. Across all sites, we detected pharmaceuticals in 50% of the spiders and in 40% of the water samples. In water, we found 60 different pharmaceuticals from 20 therapeutic drug classes, the most frequent were antihypertensives, antibiotics, and antidepressants. Similarly, in spiders, we detected 59 compounds from 15 classes mostly antihypertensive, anxiolytics, and antidepressants. The compounds with the highest concentrations in water were the stimulant caffeine, and the analgesics paracetamol and codeine. In spiders, the most common were the analgesic paracetamol, antibiotic trimethoprim, and the corticosteroid beclomethasone. We found 44 compounds shared between spiders and water samples, most of which were quantified in concentrations significantly higher in spiders, as much as 1000 times greater. The detection of a diverse range of pharmaceuticals, including some at concentrations significantly elevated in spiders compared to water, underscores the potential for biomagnification within the aquatic-terrestrial interface. Our study not only reveals the widespread exposure of aquatic and riparian organisms to pharmaceuticals but also highlights the need for further investigation on the pathways through which contaminants move across ecosystems and the potential risks this poses to wildlife.

### 3.20.P-Th355 Assessing the environmental fate of novel peptidomimetic antimicrobial molecules

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Antibiotics are molecules used to treat bacterial infections that have led to major improvements in human and veterinary medicine. However, the overuses and misuses of antibiotics caused the development of antimicrobial resistance (AMR). Due to poor absorption, large amounts of antibiotics and transformation products (TP) are excreted and can be emitted in the environment, either directly or *via* wastewater treatment plants effluents. In the environment those compounds impact both human and environmental health by contributing to the spread of AMR and affecting natural bacterial communities. There is therefore an urgent need of innovative molecules that are effective against antimicrobial-resistant bacteria and safe for the environment. Antimicrobial peptides (AMP) are antibiotics molecules naturally produced by organism for defending against infectious disease. Peptidomimetic compounds are derived from AMP and mimic their antimicrobial activities but without their drawbacks (toxicity, poor stability). 4 peptidomimetics molecules have recently been synthesized from *S. aureus* PepA1 toxin. These new compounds are cyclic pseudopeptides (Pep) and could be good candidates for clinical evaluation. Therefore several of their characteristics need to be evaluated, including their environmental fate and impacts on microbial communities. Firstly, natural attenuation processes (i.e. hydrolysis, photolysis, biodegradation, adsorption) are assessed in batch scale. These controlled experimental conditions allow to isolate each process and thus to understand their kinetics under various environmental conditions, their degradation pathways and major TP. Peps concentrations are measured by HPLC-MS/MS whereas degradation pathways and transformations products are characterized by HPLC-HRMS. As Peps are huge molecules (>1000 g/mol), TP could be toxic and more stable than their parents as it is the case for vancomycin, another AMP. It is therefore important to characterize Peps TP too. However, batch conditions are simple and poorly represent environmental fate of chemicals. Consequently a mesocosm experimentation will be carried out. Results obtained in batch will be used to develop an appropriate methodology and to simplify those obtained in mesocosms. The combination of batch and mesocosms will allow to understand Peps environmental fate and impacts on microbial communities, thereby reducing environmental and health risks if those compounds are commercialized.

### 3.20.P-Th356 Biosorption of Pharmaceutical Compounds under Acidogenic and Methanogenic Condition during Anaerobic Digestion: Effect of pH and Glycerol Co-fermentation

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Biosorption and biodegradation have been identified as the main mechanisms for removing pharmaceuticals compounds (PhCs) in wastewater treatment plants. Depending on the compound, biosorption on sludge can represent a relevant factor in its removal, and according to its magnitude, it can represent a potential risk when sludge is disposed in the environment. Sorption behavior can be estimated with the sorption coefficient ( $K_D$ ), which is dependent on the characteristics of the contaminant and the type of sludge. This work aimed to evaluate the biosorption of different PhCs in a two-phase (acidogenic-methanogenic) anaerobic bioreactor. The reactor operation was divided into two operational phases: the first treating lab-made sewage (169 days) and the second using glycerol (GOH) as co-substrate (80 days). PhCs belonging to different classes were selected: the antibiotic sulfamethoxazole (SMX); the anti-inflammatory drugs naproxen (NPX) and diclofenac (DCF); the analgesic acetaminophen (ACT); the beta-blocker metoprolol (MTP); and the antiepileptic carbamazepine (CBZ). Samples of acidogenic and methanogenic biomass were removed from the reactor and PhCs were extracted from the biofilm by ultrasonic solvent extraction with methanol and acetonitrile, prior to the solid phase extraction and analysis by liquid chromatography coupled with mass spectrometry. As main results, three types of PhCs biosorption behavior were observed. SMX and ACT were not detected in the acidogenic and methanogenic sludge as they are more hydrophilic (low  $\log D_{ow}$  at any pH). CBZ and MTP showed low sorption - low  $K_D$  values (71 – 149 and 23 – 76 L kg<sup>-1</sup>TSS for CBZ and MTP, respectively), also evidenced by the  $\log D_{ow}$  values that do not vary with the decrease in pH in the acidogenic reactor. Finally, NPX and DCF showed moderate to high sorption and were influenced by the pH of the medium. With the GOH co-digestion, the acidogenic reactor pH decreased from 5.5 to 4.2, increasing  $\log D_{ow}$  of these compounds and, thus, influencing biosorption, also demonstrated by the  $K_D$  values in the acidogenic reactor (NPX: 229 – 1843, DCF: 702 – 5950 L kg<sup>-1</sup>TSS, without and with GOH, respectively). The results proved that acidogenesis and methanogenesis act differently on the PhCs biosorption. The GOH co-fermentation acted specifically on the biosorption of NPX and DCF, due to the lowering of the pH by the organic acids production.

### 3.20.P-Th357 Degradation Studies of Some Pharmaceuticals Present in Sewage Sludge

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Soil pollution has a clear impact on food quality. At the same time the global deficit of fertilizers is present and due to the war in Ukraine the situation is worsening. Still, a number of possible resources of nutrients have not found appropriate application in agriculture, as for example sewage sludge (SS), which is one of the most promising sources of P, N and organic matter. SS usage as an agricultural fertilizer has a number of limitations. Amongst them persistent organic pollutants (POPs) have a special role due to their ability to undergo plant uptake and *via* this affect living organisms.

Intensive studies involving the fate and possible means of eliminating POPs present in SS are going on, but due to the complexity of these compounds no major success has been achieved. In some cases promising results have been gained *via* SS composting with different bulking agents. Our work has been concentrated on studying the degradation of several widely used

POPs, as for example pharmaceuticals, during composting. We have shown, that the degradation rate of pharmaceuticals and personal care products, as in the case of carbamazepine (CBZ) and triclosan (TCS), depends on composting conditions.

### **3.20.P-Th358 Unravelling the Dynamics of a Photolabile Pharmaceutical in the River Rhine: Real-Life Degradation Rates and the Impact of Sunlight Variability**

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Pharmaceutical contaminants, including the photolabile diclofenac (DCF), pose a risk to the environment in rivers and streams, sometimes exceeding proposed standards. However, the lack of real-world degradation constants creates a knowledge gap that hinders effective management. Accurate numbers from river conditions are lacking, despite extensive laboratory research. Our study addresses this issue by developing a method to determine the degradation rate constants of the DCF using high-resolution monitoring data from rivers. Routine monthly monitoring is insufficient due to the rapid turnover of DCF, which requires higher temporal resolution. Through near daily non-target screening (NTS) data integration and modelling, our goal is to calculate degradation rate constants and assess seasonal and annual changes in DCF load.

We first calibrate the daily NTS data to monthly DCF concentration monitoring to ensure accuracy and reliability. With the calibrated NTS data, we modelled DCF load to reconstruct the DCF time series by assuming first-order degradation based on sunshine duration and discharge. We successfully reproduce the weekly to seasonal variations of DCF concentration at two Rhine stations (km 359 and km 590). Our calculated degradation rate constant ( $0.14 \text{ h}^{-1}$ ) agrees with laboratory studies, and with this calculation we extended the temporal coverage of the daily DCF concentration to 2012. Comparing the annual variation in DCF load from both data and model to the variation in sunlight, we find that more than 63% of the DCF load variation is due to the variation in sunlight.

In conclusion, the half-life of DCF in our study area is approximately 5 hours. Sunlight changes dominate DCF load variations over the last decade, overshadowing changes in consumption patterns. The approach will be extended to other photolabile substances and integrated into river hydrodynamic models for broader spatial coverage.

### **3.20.P-Th359 Pharmaceutically Active Compounds (PhACs) in sediments and fish from a fluvial ecosystem. Tagus River case study**

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The presence of pharmaceutically active compounds (PhACs) in river waters is well established, but their mobility to other compartments may endanger the quality and health of the whole ecosystem. The present study addressed the presence of 22 PhACs and one metabolite in river sediments (n=28) and fish samples (n=24) collected from the Tagus River watershed during 2020, 2021, and 2022.

PhACs were analyzed in both matrices by an adapted QuEChERS extraction method and SPE clean-up step. Instrumental analysis was carried out by ultrahigh performance liquid chromatography tandem mass spectrometry (UPLC-TqMS/MS) followed by quantification with deuterated standards.

For the validation of the analytical method for both matrices, samples with no PhAC levels were processed as previously described. Limits of quantifications meeting SANTE/2020/12830 and SANTE/11312/2021 criteria ranged between 1 to 50 ng/g for sediments and between 10 to 50 ng/g dry weight (dw) for fish. 9 PhACs were quantified in at least one sediment sample while only clotrimazole PhAC was found above LOQ in fish. Quantification frequencies were higher than 50% for azithromycin, clarithromycin, clotrimazole, irbesartan, miconazole, o-desmethylvenlafaxine, and venlafaxine in sediments. No temporal (2020-2021-2022) tendencies ( $p > 0.05$ : Kruskal-Wallis test) were found. According to results obtained in surface water sampled in the same period, areas with the highest PhACs concentration in river water showed the highest PhAC levels in sediments and biota, revealing a possible mobility between these environmental compartments. In addition, PhACs with elevated  $K_{ow}$  and  $K_{oc}$  values as clotrimazole were found in both matrices with a median concentration of 20 ng/g in sediments and 12 ng/g dw in fish samples.

In accordance with the proposal for amending the Water Framework Directive, five PhACs will be included as Priority Substances. However, environmental quality standards (EQS) are not defined for sediment and biota for either of them. Nevertheless, the proposal identifies azithromycin and clarithromycin as substances that tend to accumulate in sediment and/or biota matching with the results of this study.

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### **3.20.P-Th360 A microcosm to elucidate the mobility of wastewater derived pharmaceuticals in soils and their effects towards respiration - a combined fate and effect study**

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The increasing adoption of wastewater reuse in agricultural irrigation, driven by climate change and water scarcity, is a sustainable solution for water management, however, inadvertently introduces a suite of emerging contaminants into soils. Contaminants including pharmaceuticals such as antibiotics, anti-depressants and anti-inflammatories are widely detected in soils following wastewater irrigation. Nevertheless, our understanding of terrestrial fate and effects of these contaminants in soils are still within their infancy. This gap is particularly critical given the potential impact of antibiotics on soil microbial communities. To address this, a combined microcosm was developed to investigate the leaching behaviour of pharmaceuticals in two arable soils (silty clay loam and a sandy loam). The microcosm includes a 30 cm soil profile and an automated system to analyse CH<sub>4</sub>, CO<sub>2</sub>, N<sub>2</sub>O, NO, and NO<sub>2</sub>; at regular time intervals; automation was provided via an Arduino chip and solenoids. Over the duration of two months, the columns were exposed to simulated irrigation events using synthetic wastewater spiked with antibiotics (azithromycin, trimethoprim, lincomycin, and sulfadiazine). Preliminary results show that exposure to environmentally realistic concentrations (100-1000 ng/L) of azithromycin, trimethoprim, lincomycin, and sulfadiazine, resulted in a 17 – 50 % increase in CO<sub>2</sub> respiration and no alterations to CH<sub>4</sub> production. Given that the system currently evaluates synergistic effects, a repeat experiment is underway to explore the effects of individual antibiotics on the production of gas emissions from soils. Furthering this leaching data will be predicted using the best available models, in the attempt to bridge gaps between current risk assessments (manure and biosolid applications) and future agricultural practices. Overall, this research offers vital insights into the terrestrial fate and effects of pharmaceuticals in agricultural soils, contributing to a more nuanced understanding of the environmental implications of using reclaimed wastewater in agriculture.

### **3.20.P-Th361 Prioritisation of fish testing requirements for human pharmaceuticals – a quantitative impact analysis**

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Among the approximate 3500 Active Pharmaceutical Ingredients (APIs) on the market in the EU many were approved for use prior to 30<sup>th</sup> October 2005 and as such were not subject to the requirement for environmental risk assessment. As part of the ongoing revision to the general pharmaceutical legislation (proposal for directive repealing direction 2001/83/EC) there is a proposal for the European Medicines Agency, in conjunction with other agencies, to prepare a risk-based prioritisation of these so-called “legacy” APIs, for provision of environmental data and risk assessments. A recent publication by Coors et al., 2023 has proposed an approach whereby a decision tree is utilised to determine if vertebrate ecotoxicity testing of pharmaceuticals is necessary. If this decision tree was implemented as part of such a risk-based prioritisation system for these legacy APIs, a significant saving of fish tested could be realised. Here we demonstrate the potential impact in terms of animals, capacity and financial savings. Using the database of APIs and ecotoxicology data availability collected during the IHI PREMIER Project, we show that implementing the approach published by Coors et al., for all APIs without fish chronic data, could save up to \$30m and 240,000 fish. Furthermore such an approach optimises capacity at contract research facilities to conduct hundreds of fish early life stage studies on other APIs allowing risk of more than 1000 legacy APIs to be clarified more quickly and using less fish. More detailed analysis will be presented showing different scenarios of different levels of prioritisation of APIs. In future, additional savings could be made through implementation of this approach for new APIs and integration of *in silico* and *in vitro* tools to further refine potential for fish sensitivity. This analysis has indicated the benefits beyond bioethics of the prioritisation of data generation activities, allowing more expedited environmental assessments and effective utilisation of vertebrate testing resource where it provides the best value and most effective environmental protection.

### **3.20.P-Th362 Ecological risk assessment of human pharmaceuticals detected in Japan and establishment of ecotoxicity database**

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Guidance for environmental risk assessment (ERA) of new human pharmaceuticals were published in 2016 by Japan Ministry of Health, Labour and Welfare with referring first version of Guideline on the environmental risk assessment of medicinal products for human use published by European Medicines Agency (EMA) in 2006 but the risk assessment is voluntary-base and the comprehensive ecological risk of pharmaceuticals have not been conducted in Japan mainly due to the lack of ecotoxicity data. Therefore, we conducted the short-term (sub-)chronic aquatic toxicity tests using three species, zebrafish (*Danio rerio*), *Ceriodaphnia dubia*, and green alga (*Raphidocelis subcapitata*) for 30+ out of 100 pharmaceuticals detected in rivers and effluent of wastewater treatment plants in Japan. Some of newly obtained data were compared with the existing acute/chronic data. Both acute/chronic ecotoxicity data were also collected from European public assessment reports (EPAR, EMA), Drugs@FDA database (FDA), pesticides ERA related materials (MOE, Japan), Safety Data Sheets (SDS) of pharmaceutical companies, and research articles. These data were curated by chemicals and toxicologists in our research group based on our expertise in ecotoxicity. 1095 test data for 363 pharmaceuticals had already been published in open database (Yamada et al., *Fundamental Toxicological Sciences*, 8(7):195-204, 2021) and additional 3309 test data for 255 pharmaceuticals are planning to be added in the database and be donated to OECD QSAR Toolbox by the end of 2024. Based on this database, the ecological risk assessment was conducted using the monitoring data of our research team in rivers and effluent of wastewater treatment plants all over Japan and the 100+ pharmaceuticals were ranked with their hazard quotient calculated by the measured environmental concentration (MEC) divided by the predicted no effect concentration (PNEC).

### 3.20.P-Th363 Ecological Risk Assessment of Individual PPCPs and Their Mixtures in Korean Surface Waters

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The ubiquitous presence of pharmaceuticals and personal care products (PPCPs) in water bodies, even at low concentrations, is concerning due to their pseudo-persistence and toxicity. This study assessed the ecological risks of individual PPCPs and their mixtures in Korean surface waters. Using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS), 137 PPCPs were monitored in four major rivers over a year, revealing concentrations from a few ng/L to 42,733 ng/L for metformin. The Risk Quotient (RQ) methodology, including an analysis of the frequency with which the Measured Environmental Concentration (MEC) exceeds the Predicted No-Effect Concentration (PNEC), referred to as  $RQ_F$ , was used to evaluate potential impacts on aquatic life. After considering the frequency of PNEC exceedance, the  $RQ_F$  values generally decreased, but clotrimazole still exhibited the high  $RQ_F$  of 17.4 with 99.6% of its MECs exceeding PNECs. The Hazard Index (HI) for PPCP mixtures was calculated using the lowest PNECs from all available aquatic organisms and three trophic levels, yielding HI values of 24.02 and 11.37, respectively. This suggests that focusing solely on three trophic levels might underestimate the ecological risks of PPCPs, especially to sensitive species like insects, which share vulnerabilities with PPCPs and pesticides. A comparison between the Maximum Cumulative Ratio (MCR) and HI revealed that the risk is primarily associated with individual PPCPs, particularly clotrimazole. Subsequently, the RQ based on the Sum of Toxic Units ( $RQ_{STU}$ ) was calculated, indicating that fish are the most sensitive group in assessing ecological risks of PPCP mixtures, with an  $RQ_{STU}$  value of 6.45. This study highlights the urgent need for comprehensive ecological risk assessments of PPCPs due to their significant threat to aquatic environments and potential impact on human health.

### 3.20.P-Th364 A biomarker-based investigation of the effects of metformin and guanylurea on the Mediterranean mussel, *Mytilus galloprovincialis*

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Metformin (MET) is a first line oral therapy for diabetic patients, and their number is expected to reach 643 million by 2030, which represents over 11% of the population between 20 and 79 years old. Accordingly, researchers have found that MET is released into aquatic bodies in higher amount than other drugs in terms of weight. MET is mainly transformed to guanylurea (GUA) and both may adversely affect organisms living in aquatic environments. Only a few data are available for marine species, thus the current study was conducted to examine the effects of MET and GUA in adult marine mussel *Mytilus galloprovincialis* after a 3-day exposure to environmentally realistic concentrations (0.5, 5 and 10 ng/L). Six biomarkers were evaluated, including lysosomal parameters (lysosomal membrane stability, LMS; lysosome to cytoplasm ratio, LYS/CYT; lipofuscin, LF; neutral lipid, NL), and oxidative stress enzyme (glutathione-S-transferases, GST and catalase, CAT) activities. Further biomarkers will be assessed, e.g. neurotoxicity stress enzyme (acetylcholinesterase, AChE) and lysosome membrane stability in digestive gland. According to the results, the highest tested concentration (10 ng/L) of MET and GUA had a significant effect on mussel LMS. Reduction in LMS is an early warning signal of damage to cells in *Mytilus* species. MET at 10 ng/L also significantly increased the accumulation of end products of lipid peroxidation (LF content). Mussels exposed to all tested concentrations of GUA showed a significant dose-dependent increase in CAT activity in both digestive gland and gills, as well as in GST activity in gills. The GST activity was also increased in digestive glands exposed to GUA, with a bell-shaped trend reaching significance at 5 ng/L. When MET was applied, CAT activity was significantly decreased in both gills and digestive gland at 10 ng/L; at the same concentration GST activity showed significant decrease only in gills. Overall, the parent compound MET has shown major effects on lysosomal parameters, meanwhile, the metabolite GUA impacted mainly the activity of oxidative stress enzymes both in gills and digestive glands of mussels. The European 4<sup>th</sup> WATCH list has prioritized MET and GUA as monitoring substances in surface waters; according to the findings of this study, more investigation is recommended on the occurrence of MET and GUA also in the marine environment, where they can affect living organisms.

### 3.20.P-Th365 Hazards of Cardiovascular Drugs in the Aquatic Environment: Impact on Growth and Vascular System Development in Zebrafish (*Danio rerio*) Embryos

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Antihypertensive drugs are a class of therapeutic agents commonly detected in the environment.  $\beta$ -Blockers, such as metoprolol, and angiotensin II-receptor blockers, such as valsartan, stand out as the most common detected cardiovascular drugs in freshwater and marine samples. The veterinary drug carazolol is a  $\beta$ -blocker that has been co-detected with metoprolol and valsartan and represents 2 % of the cardiovascular drugs found in environmental samples. While few studies have described the effects of metoprolol in fish and little data have been reported for valsartan, no data is available on the (eco)toxicity of carazolol. In the present study, some knowledge gaps about the (eco)toxicity of metoprolol, valsartan and carazolol are filled by data obtained from experiments on the zebrafish embryo model.

For all compounds, the Fish Embryo Acute Toxicity (FET, OECD 236) test was conducted in 3 replicates with wild-type zebrafish (*Danio rerio*) Embryos. Teratogenic effects were recorded and the median lethal concentration (LC50) and median effect concentration (EC50) were calculated. Growth and heart-beat rate were considered as additional endpoints. Effects on the development of the vascular system were observed through the transgenic *Danio rerio* Tg(kdrl:GFP) strain. Vascular

system malformations were analysed at 120 hpf through fluorescence microscopy. Preliminary results of metoprolol showed 50 % lethality at 700 mg/L, 120 hpf. At  $\geq 300$  mg/L, hatched embryos presented scoliosis and lordosis of the spine and motility decreased to 0. Measurements of the body length of hatched larvae revealed a trend of inhibition in growth ( $\geq 500$  mg/L). At 72 hpf, individuals exposed to  $\geq 300$  mg/L of metoprolol showed cardiac effects. Preliminary morphological observations on the Tg(kdrl:GFP) line did not reveal any obvious malformations on the blood vessels at 120 hpf.

Subsequent tests will shed light on the (eco)toxicity of carazolol and provide standardised data through the execution of FET tests on valsartan and metoprolol. Effects on growth, behaviour, as well as endocrine and antioxidant systems caused by cardiovascular drugs have been reported in non-target organisms. So, it is indispensable to produce base evidence of ecotoxicity through established in vivo models. Experiments with the vascular fluorescent transgenic line Tg(kdrl:GFP) will show its effectiveness as an ecotoxicological tool for screening drugs for their potential to impair the vascular system development.

### **3.20.P-Th366 Toxicity of Diclofenac Towards Baltic Cyanobacteria and Their Biochemical Response**

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Cyanobacteria occurring in the Baltic Sea are constantly exposed to the pharmaceutical contaminants. One of the pharmaceuticals detected in the Baltic Sea that is of particular concern is diclofenac - DCF. Cyanobacteria play an important role in the ecosystem and significantly affect the condition of entire water reservoirs. On the one hand, they constitute the basis of the food chain and produce oxygen in quantities significant for the entire globe. On the other hand, they create problematic blooms during which they release toxins that are harmful to humans and animals. Increased blooms may cause hypoxia and deteriorate the condition of ecosystems, and ultimately lead to eutrophication of the entire water reservoir. The aim of this study is to investigate the impact of DCF on several different species of cyanobacteria occurring in the Baltic Sea. The study took into account not only the effect of this pharmaceutical on the growth of cyanobacteria in reference ecotoxicological tests, but also determined other endpoints, such as changes in the profile of photosynthetic pigments and toxins produced by them, observation of changes in the size and number of cells using flow cytometry, and growth assessment by optical density. The release of reactive oxygen species in response to stress caused by the presence of DCF has also been studied. The results showed high resistance of unicellular cyanobacterial species *Synechocystis salina* and *Microcystis aeruginosa* in reference ecotoxicological tests. In turn, according to preliminary tests, multicellular cyanobacteria with a filamentous structure were more sensitive; their growth was inhibited at a DCF concentration of several mg L<sup>-1</sup>. In some of the tested cyanobacterial cells, despite no effect on cell growth, a biochemical response was observed, i.e. increased secretion of toxins or an effect on photosynthesis.

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### **3.20.P-Th367 Pharmaceutical Pollution Drives Changes in the Composition and the Functionality of the Aquatic Microbial Biotic and Abiotic Communities**

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Pollution is a worrying problem today that affects all ecosystems. Aquatic ecosystems are among the most affected as they are a sink for pollutants from various sources. Among the main chemical pollutants threatening the stability of aquatic ecosystems, pharmaceuticals stand out due to their exponential increase in use for both human treatment and intensive livestock farming. As environments are exposed to multiple stressors, we have used multispecies assays, including the bivalve *Scrobicularia plana*, in artificial controlled microcosms to assess the specific impact of different pharmacological compounds on the microbiome. Their effects on both the abiotic and biotic matrices of the ecosystems were analyzed using metagenomic techniques. We used 16S rDNA sequencing to determine changes in the structure, diversity, and functionality of bacterial populations from sediments and the digestive gland of bivalve molluscs. Here we present the effects of the drugs carbamazepine (CBZ), an anticonvulsant used to treat epilepsy and bipolar disorder, and sulfamethazine (SMZ), a synthetic antibiotic of the sulfonamide family, when exposed individually and in combination to a concentration of 1  $\mu$ g/L, which is an environmentally relevant concentration, for 10 days. An unexposed control was also included. Overall, the phylum with the highest number of identifications in all samples was Proteobacteria. The families Ectothiorhodospiraceae and Flavobacteriaceae were highly represented in both types of samples. Exposure to SMZ resulted in a significant decrease in families related to nitrogen fixation, such as Thioalkalispiraceae and Xanthomonadaceae. In addition, CBZ had an antagonistic effect on SMZ. At the functional level, there was a significant decrease in "anoxygenic photoautotrophism" after single exposure to SMZ compared to the control. The changes observed in the microbiome can be used as biomarkers of environmental exposure to these drugs.



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### **3.20.P-Th368 Green microalgae respond differently to the toxicity of diclofenac. An study with *Scenedesmus quadricauda* and *Ankistrodesmus falcatus***

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Each year, 1443±58 tons of diclofenac are consumed worldwide. This non-steroidal anti-inflammatory drug's high consumption and incomplete metabolism have caused it to be widely distributed in aquatic environments, posing a risk to microalgae and ecosystems. This study aimed to evaluate the effect of diclofenac on population growth, in the concentration of photosynthetic pigments, and the main biomolecule composition in *Scenedesmus quadricauda* and *Ankistrodesmus falcatus*. Bioassays were performed in PCG medium for *S. quadricauda* and Bold's Basal Medium for *A. falcatus* with 0.1, 1, 10, and 100 mg L<sup>-1</sup> diclofenac, incubating with constant aeration and continuous illumination at 25°C for 96 hours. The population growth of *A. falcatus* was inhibited by 43, 55, and 99% with 1, 10, and 100 mg L<sup>-1</sup> diclofenac, respectively, while *S. quadricauda* showed no significant growth change at any drug concentration. However, in *S. quadricauda*, an increased frequency in atypical cells was observed as the concentration of diclofenac was augmented. In *A. falcatus*, the concentration of carotenoids and chlorophylls *a* and *b* increased significantly with 1 and 10 mg L<sup>-1</sup> diclofenac, respectively, compared with the control. Lipid concentration in *A. falcatus* exposed to all concentrations of diclofenac was higher than the control, and proteins increased by 152 and 217% with 1 and 10 mg L<sup>-1</sup>, respectively; carbohydrates increased by 190% with 10 mg L<sup>-1</sup> diclofenac. For *S. quadricauda* exposed to diclofenac, the concentration of chlorophylls *a* and *b* was similar to that determined in the control. However, carotenoid and lipid content decreased significantly in all drug concentrations, and the protein concentration increased by 271, 338, and 339 %, respectively, for the concentrations of 1, 10, and 100 mg L<sup>-1</sup>. In comparison, carbohydrates increased by 204 and 207% with 10 and 100 mg L<sup>-1</sup> of diclofenac. The results confirm the adverse effects of diclofenac on primary producers despite the differences in toxic responses. It was also evidenced that different endpoints should be studied in aquatic biota for a complete assessment of drugs toxicity as emerging concern pollutants.

### **3.20.P-Th369 Does DCF influence antioxidant enzyme system in Baltic cyanobacteria? Biochemical response of the cells to environmental emergent pollutant**

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The presence of pharmaceuticals in the environment is a global challenge. Diclofenac (DCF) is a pharmaceutical which deserves special attention due to its low removal rate in wastewater treatment plants (WWTPs), the high frequency of its detection in natural waters and proven negative impact in low concentrations on non-target wild-living organisms.

The Baltic Sea, is a diverse, closed and shallow basin, and is one of the most polluted seas by various anthropogenic compounds. Enhanced eutrophication is one of the causes of the formation of cyanobacteria blooms. Cyanobacteria, just like other organisms occurring in the Baltic Sea, are chronically exposed to pharmaceuticals including DCF. Currently, there is limited knowledge on the pharmaceutical effect on cyanobacteria, while some works are available on selected green algae and diatoms. Available literature data indicate higher resistance of cyanobacteria to pharmaceuticals compared to, for example, green algae, due to their overall high adaptive possibilities. The study of the impact of pharmaceuticals on phytoplankton focuses mostly on the growth inhibition endpoint in acute studies, which gives information about the general toxicity but not about biochemical changes occurring in cells.

Taking all this into account, the aim of the research was primarily characterization of the antioxidant enzyme system in *Synechocystis salina*, *Microcystis aeruginosa*, *Nodularia spumigena*, *Aphanizomenon flos-aquae* - the dominant species of cyanobacteria occurring in the Gulf of Gdańsk, the Polish part of the Baltic Sea. The presence of enzymatic activity of glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and peroxidases (GPx) was examined. The antioxidant enzyme system of target cyanobacteria species was never previously described. As the Baltic cyanobacteria are chronically exposed to DCF present in the environment, the biochemical response (induction/inhibition of selected antioxidant enzymes) of the cells was studied.

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### **3.20.P-Th370 Application of flow cytometry for testing of pharmaceuticals toxicity toward cyanobacteria**

Klaudia Kwizdzińska, Katarzyna Bethke and **Magda Caban**, Department of Environmental Analysis, University of Gdansk, Poland

Pharmaceuticals are among the challenges of modern ecotoxicology. Three decades of its monitoring shows that they are present in all water types. The huge number of pharmaceuticals, its diverse characteristics and mode of action cause problems with testing of toxic effect on water organism, which also are represented with thousands of species. Thereby, the reference test organisms are taken for ecotoxicology. The water microorganisms are mostly represented by green algae. Cyanobacteria are much less often the targets, despite their high impact on the environment caused by blooms creation.

In this work we decided to present the application of flow cytometry for testing the cell number during test of pharmaceuticals to cyanobacteria. The *Synechocystis salina* and *Microcystis aeruginosa* were taken as representants of unicellular cyanobacteria. The ampicillin antibiotic was taken as target and acute 96h test was performed. This substance was found to have EC50 lower than 10 mg/L. The performance of flow cytometry measurement shows that despite the cell number the results of this analysis can show how the basic parameters and cell characteristic can be changed by toxicants. Preliminary studies with staining by fluorescein diacetate were performed. The correlation between cell number and optical density shows perfect agreement, what mean that they can be alternatives. As conclusion, the advantages and disadvantages of flow cytometry for unicellular cyanobacteria counting was presented.

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### **3.20.P-Th371 Antibiotics and its Associated Risk in an Indonesian Reservoir**

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While various classes of antibiotics have been detected in rivers and reservoirs worldwide, there is limited knowledge of antibiotic occurrence and their risk in the surface waters of Southeast Asia. This is due to the unavailability of data on consumption of antibiotics and a lack of environmental monitoring. This study aims to provide insight into the occurrence and ecological risk associated with various classes of antibiotics in the Cirata reservoir in Indonesia. For this, we analyzed 65 types of antibiotic residues including the antibiotic classes of tetracyclines, fluoroquinolones, macrolides, sulfonamides, lincosamides, diaminopyrimidines, and amphenicols in water and sediment samples. Our assessment of wet-season samples shows that at least 14 types of antibiotic residues with concentrations ranging from below the limit of quantitation to hundreds of ng/L were present in the water samples. Also, a relatively high concentrations ( $\mu\text{g}/\text{kg}$ ) of 5 types of antibiotic residues were present in the sediment. The individual preliminary risk assessment of the highest antibiotics concentration present in the water and sediment samples shows that fluoroquinolones and tetracyclines poses critical risk for the prevalence of antimicrobial resistance development. Meanwhile, their risk to the aquatic organism such as phytoplankton and crustacean is unlikely. Further assessment on the mixture of antibiotic residues in relation to ecological risk should be performed to provide a more comprehensive impact. The information provided in this study is helpful to define further ecological impact of antibiotics on the supporting services provided by the reservoir, the sources, and effective strategies for dealing with ecological impacts and antimicrobial resistance on local as well as regional scales.

### **3.20.P-Th372 Pharmaceutical pollution in agriculture: Impacts & risks of antidepressants on soil health and crop production**

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Recent environmental concerns regarding pharmaceutical pollution have primarily focused on its impact on aquatic ecosystems, neglecting its effects on agricultural soils. The heightened use of reclaimed wastewater and biosolids in agriculture intensifies soil exposure to pharmaceuticals. Given the increasing reliance on reclaimed wastewater due to climate change, urgent research is imperative to understand the chronic, low-level exposure of agricultural soils to pharmaceuticals. This comprehension is vital for informing environmental risk assessments, guiding land management practices, and addressing the role of reclaimed wastewater in climate change adaptation. Limited research exists on the impacts of pharmaceuticals on soil, microbial communities, and crops. This review aims to assess the current knowledge on contaminants of emerging concern (CEC), with a specific focus on antidepressants. The investigation will evaluate both predicted and measured effects of individual pharmaceuticals and their mixtures on soil health, microbial communities, and crops.

This literature review will delve into the abundance, types, and levels of antidepressants found in agricultural systems, considering the diverse range of pharmaceuticals in circulation including, but not limited to, sertraline, venlafaxine, citalopram and fluoxetine. Current studies predominantly focus on antidepressant impacts in aquatic systems, and so the basis of the extent of terrestrial exposure will build from this. The study will explore the routes and mechanisms through which antidepressants may cause adverse effects on various ecosystem processes and compartments, including crop health, soil microbial processes, and the physical and chemical properties of the soil. Emphasis will also be placed on the acknowledgement and assessment of CEC mixtures, as well as the individual risk of the chemical on the environment. Through this literature review, we aim to contribute to the advancement of knowledge on pharmaceutical pollution in agricultural systems, assessing the potential challenges and risks posed by antidepressant contamination.

### **3.20.P-Th373 Are there alternatives to PFAS pharmaceuticals?**

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The national authorities of Denmark, Germany, the Netherlands, Norway, and Sweden are currently proposing a comprehensive restriction covering a wide range of perfluorinated and polyfluorinated alkyl compounds (PFAS). By definition

(fluorinated methyl (CF<sub>3</sub>-) or methylene (-CF<sub>2</sub>-) carbon atom without any H/Cl/Br/I atom attached to it), some active pharmaceutical ingredients (APIs) in human and veterinary medicinal products (HMP/VMP) can also be categorised as PFAS. Exemptions from the restriction should only be provided for uses that are considered necessary for health, safety or are critical for the functioning of society.

Many medicinal products are necessary for human and animal health and it is, therefore, likely that they will be exempted from the restriction. This means, other ways to reduce the use of PFAS-APIs need to be considered. Often, there are several APIs for the same indication. For example, the *World Health Organisation's Model List of Essential Medicines* lists therapeutic alternatives for numerous APIs, to be used if the API recommended in the first instance is not readily available for certain reasons. The DrugBank databank also offers a service to identify potential therapeutic alternatives.

We have identified 70 approved PFAS APIs in the DrugBank. For the majority of these, non-PFAS alternatives are available. How they and their alternatives were identified and some prominent examples will be presented on the poster. The second aim of the project was to identify which PFAS-types (e.g. CF<sub>2</sub> or CF<sub>3</sub>) are present and what are the reasons for the fluorination. The majority of the PFAS-APIs contain a CF<sub>3</sub> group (84 %), which is remarkable as the CF<sub>3</sub> group is a terminal group and often not directly involved in binding to the active site. The final degradation product of most of the identified CF<sub>3</sub> groups is trifluoroacetic acid (TFA), an already nearly ubiquitous anthropogenic substance found in almost all compartments, even in rain and drinking water. Due to the existence of non-PFAS alternatives for the majority of the PFAS-APIs investigated, the inclusion of CF<sub>3</sub> in APIs in the current scale is questionable.

The results can be used by researchers involved in drug discovery to reconsider whether the inclusion of the CF<sub>3</sub> group is really necessary for the functionality of the drug, but can also be used by doctors and pharmacists to select more sustainable pharmaceuticals.

### **3.20.P-Th374 Demonstrating the environmental benefits of greener alternative pharmaceuticals in a hazard driven regulatory landscape**

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Pharmaceuticals have a critical role in the health and well-being of society, providing lifesaving treatments, helping patients to manage long term health conditions, and enhancing general quality of life. There are however environmental risks associated with increasing concentrations of pharmaceuticals in the environment (PiE). Recognising this, in March 2019 the European Commission (EC) published a Strategic Approach comprising multiple initiatives to address pharmaceutical pollution ranging from regulatory proposals aimed at controlling or managing emissions (i.e., end of pipe measures), to more proactive approaches to reduce input or adverse effects at source by designing pharmaceuticals which have a more favourable environmental profile, the “Greener Pharmaceutical” concept. A problem with the Strategic Approach is that hazard-based approaches aimed at reducing pharmaceutical emissions are not aligned with promoting innovation of greener alternative pharmaceuticals. This is demonstrated through a case study with the novel natural oestrogen estetrol (E4) which has been shown to be a greener alternative to 17 $\alpha$ -ethinyloestradiol (EE2) for use as a human contraceptive. E4 is demonstrated as having a more favourable environmental profile compared with EE2 (being less persistent and having lower potential for bioconcentration) and risk quotients (RQ) calculated according to European Medicine Agency (EMA) guidelines are <1 for use as a contraceptive (compared with RQ of 3.9 for EE2 in an equivalent product). Nevertheless, the lower potency of E4 for the oestrogen receptor means higher doses are prescribed with the potential for higher environmental concentrations despite a lower risk for aquatic organisms. For E4, conservative Predicted Environmental Concentration (PEC) in surface water are up to 31 ng/L (i.e. not accounting for removal in the Sewage Treatment Plant), compared with 0.066 ng/L for EE2. This is a concern based on EC proposals for generic environmental quality standards (EQS) of 25 ng/L for total pharmaceutical concentrations in groundwater. We therefore recommend that potency is a consideration for developing generic EQS for pharmaceuticals.

### **3.20.P-Th375 Pharmaceuticals Strategy for Europe: A Call for Continuous Improvement towards Environmental Protection**

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In 2020, the European Commission (EC) launched its *Pharmaceutical Strategy for Europe*, with broad goals to ensure equitable access to affordable medicines, support competitiveness, innovation and sustainability, enhance crisis preparedness, and ensure the EU has a strong voice in the world. A key step in its implementation was the acceptance by the EC of a draft Regulation and Directive in April 2023, for which a stakeholder consultation period has, in November 2023, just ended. This legislation will ultimately replace the current European Medicines Agency regulation, which has seen little update since publication in 2006. Broadly speaking, its aims align with that of the Pharmaceutical Strategy for Europe, with explicit reference to aspects such as environmental sustainability and anti-microbial resistance. In this poster presentation, we will highlight enhancements in environmental protection offered by this legislation and reflect on areas where there are still gaps and room for improvement.

Examples of enhancements include a requirement to consider the whole lifecycle of products, the ability for authorities to refuse market authorisation based on the risk of environmental harm, the requirement for retrospective risk assessment of products already on the market, and the requirement for continually updated risk assessments. In addition, manufacturers must flag when substances are PBT (persistent, bio-accumulative, toxic), vPvB (very persistent, very bio-accumulative), PMT (persistent, mobile, toxic), vPvM (very persistent, very mobile) or endocrine disrupting.

Despite this enhanced protection, there are important aspects that are not addressed. For example, the risk posed by chemical mixtures, which could be included through the development of a robust mixtures assessment framework. Whilst endocrine disruption is explicitly mentioned, there is room for improvement in testing for other sub-lethal effects, such as neurotoxicity (affecting behaviour), immune modulation and genotoxicity leading to inter- and trans-generational effects. In general, environmental heterogeneity (in space and time) is not accounted for, requiring the better use of data on the effects of, for example, pH and DOC on bioavailability and climatic scenarios on temporal dynamics. Integration of these aspects would lead to better understanding, supporting strengthened environmental protection for pharmaceutical products.

### **3.20.P-Th376 Proposal for of a threshold approach to PBT/PMT assessment of active pharmaceutical ingredients (APIs)**

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The EU Commission proposal for a new EU pharmaceutical legislation foresees the need to evaluate PBT (including vPvB) and PMT (including vPvM) criteria for active pharmaceutical ingredients (APIs), and considers possible risk mitigation measures associated with these criteria. According to the current EMA guidance, PBT criteria are required to be evaluated regardless of the predicted environmental concentrations (PECs). However, consumption of different APIs and hence the respective PECs range widely. At one end, APIs are used at extremely low daily doses of few µg/day and/or for rare diseases. At the other end of the range, APIs are administered at a level of g/day and/or to very large patient populations. The PBT/PMT criteria (under REACH and CLP) are useful for identifying substances of heightened concern when similar levels of release to the environment are compared, but may not when these differ by many orders of magnitude.

Under current EMA guidance, PBT screening of APIs generally stops after the generation of log D<sub>ow</sub> data, as the action trigger of log D<sub>ow</sub> >4.5 is rarely exceeded. In the absence of any corresponding action trigger, future PMT screening would require adsorption/desorption studies (and sometimes also sediment/soil transformation tests) to be conducted for all APIs, including those that do not reach the current, exposure-based action limit (PEC = 0.01 µg/L) for Environmental Risk Assessments (ERA). Both study types are costly and require the use of radiolabeled material.

We present science-based options to establish PEC thresholds to trigger PBT and/or PMT screening. The proposed approach is conservatively designed to ensure that any potential risks a) of secondary poisoning (main concern for PBT substances) and b) to groundwater/drinking water (main concern for PMT substances) will not be missed.

Secondary poisoning risks were evaluated following ECHA guidance documents for hypothetical APIs with reasonably extreme bioaccumulation factors, toxicity, and K<sub>OC</sub>. Similarly, groundwater risks were evaluated following the draft EMA ERA guideline (2018) for hypothetical APIs with reasonably extreme K<sub>d</sub> sludge/soil values and DT50 values. Different PEC triggers and PNEC<sub>oral</sub> values were used.

The results of these simulations support that the current exposure action limit is an appropriate trigger for PMT assessment, while for PBT assessment, we propose a combined trigger based on exposure levels and mammalian toxicity data.

### **3.20.P-Th377 Toward a greener pharmacy: preparation of factsheets on potential environmental risks of pharmaceuticals products to support the environmentally conscious prescription of medicines**

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In recent decades, the combination of "pharmaceuticals" and "personal care products" (PPCPs) have been recognized as contaminants of "emerging concern" due to their persistent presence in the environment.

Many PPCPs are rapidly dispersed in the environment, but their extensive and universal use determines a pseudo-persistence in aquatic and soil environments with serious ecological impacts on aquatic organisms, thus also posing a risk of selective pressure that can lead to a reduction in biodiversity. The concern for the detection of PPCPs in the environment derives from their ubiquitous presence in bodies of water, soil, and biota.

For Active Pharmaceutical Ingredients (APIs), the concern is greater for some of their peculiar characteristics. They are substances designed to act at very low doses, to "last" for a long time, with considerable stability that limits their biodegradability and for this reason they persist for quite a long time in the environment.

In this project 300 compounds used in pharmaceutical products (such as APIs, preservatives, artificial sweeteners, dyes) will be searched in water samples. Data from the literature about their persistence, toxicity and bioaccumulation will be collected. In some specific cases, for substances considered particularly relevant, for which ecotoxicity data are not present, experimental studies will be carried out. All the data will be organized in technical sheet reporting a calculated a new presumed level of potential environmental risk. This database of concise fact sheets of PPCPs will be consulted on the web or via App for mobile devices by health professionals. The database can enable doctors, pharmacists and veterinarian, while ensuring that every patient gets the treatment they deserve, to also maintain the focus on the protection of the environment and biodiversity. Several educational initiatives will be implemented to spread the evidence that will emerge by this project which will be also open to researchers and academics of various disciplines. This work has been funded by the European Union - Next Generation EU program. PNRR, Mission 4, Component 2, Investment 1.4 - National Biodiversity Future Center (NBFC) – CUP n° B63D21015220004.

### **3.20.P-Th378 Use Pharmaceutical PNECs with Caution**

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In recent years, substantial effort has been made to measure concentrations of pharmaceuticals in the aquatic environment. By comparing measured environmental concentrations (MECs) to predicted no effect concentrations (PNECs), locales at risk from pharmaceutical micropollutants can be identified. However, not all PNECs are created equal. There are several published or presented assessments where poorly-derived PNECs were used to identify risks from MECs. PNECs considered poorly-derived include those based on predicted toxicity when empirical data is available or those based on unreliable ecotoxicity data. Examples are presented and discussed, including some reported PNECs that are orders of magnitude different from PNECs based on high quality data. Researchers should critically evaluate PNECs before using them to determine whether an MEC is safe or a concern. Elements of that critical evaluation are discussed. PNECs for pharmaceuticals should be derived using standard methodology (e.g., EPA Water Quality Standards Methodology or EU Water Framework Directive) using the best available, empirical, most recent, and preferably chronic ecotoxicity data. It is recognized that there is currently not a comprehensive database with ecotoxicity data on pharmaceuticals and not all pharmaceuticals have ecotoxicity data. Available sources for data to develop PNECs will be presented. Finally, when PNECs are used to identify risks, their origin and basis should be transparent. Only high quality PNECs should be used to make decisions by risk assessors and policy makers.

### **3.20.P-Th379 Towards a Greener Pharmaceutical. A Comparative Study of the Environmental Risk of Ciprofloxacin and its Alternative CIP-Hemi from Human to River.**

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Ciprofloxacin (CIP) stands out as one of the most extensively used fluoroquinolones globally, used for its diverse antibiotic applications against both gram-negative and some gram-positive bacterial infections. Despite its established medical efficacy, the release of ciprofloxacin into the natural environment raises significant concerns due to its slow biodegradation and the potential for fostering antibiotic-resistant bacteria. Attempts have been made to explore chemical alternatives to replace ciprofloxacin, aiming for comparable medical effectiveness with reduced environmental impacts, based on the safe and sustainable by design concept. This research seeks to conduct a comparative study on the environmental risk posed by CIP and its derivative, CIP-Hemi, from patient to river. Given several data gaps in this pathway, particularly concerning CIP-Hemi, toxicity tests were executed on cyanobacterium *Microcystis aeruginosa* following the ozonation and photodegradation experiments. These experiments aim to assess the influences of the above-mentioned processes on the ecotoxicity of CIP and CIP-Hemi. The mixtures of daughter molecules resulting from these antibiotics after each process will be examined, noting that either of the tested processes leads to the complete mineralization of these compounds. Regarding the remaining stages, information is compiled through an extensive literature review. Due to similarities in the chemical structure between CIP and CIP-Hemi, some data regarding the toxicity of CIP can be extrapolated to CIP-Hemi, an approach that was utilized for the remaining stages. The previously mentioned experiments and the literature review will be used to assess and compare the risks of CIP and CIP-Hemi.

### **3.20.P-Th380 Ecological Impacts of the Pharmaceutical Pollutant Oxazepam on Roach (*Rutilus rutilus*) Behavior in Natural Environments: Integrating Landscape Use and Resource Selection**

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Effects of pharmaceutical contaminants on non-target organisms, such as fish, have been widely reported particularly for commonly prescribed anxiolytics like benzodiazepines. Oxazepam, for example, has been shown to alter fish behavior and physiology, including activity patterns, foraging, and predator-prey interactions. While laboratory-based studies have provided initial insights into the effects of oxazepam on fish behavior, research in natural field settings is essential for assessing the ecological relevance of these findings. Here, we evaluated the effects of an environmentally relevant concentration of

oxazepam on roach (*Rutilus rutilus*) behavior, in a natural environment, combining cutting-edge approaches that increase the ecological realism of the study. We used a slow-release internal implant to deliver the pharmaceutical, and high-resolution acoustic telemetry to track fish movement in the system. We evaluated changes in roach swimming activity, activity patterns, and habitat selection. We found that exposed fish were active 50 % more time than control fish, although their diel activity patterns were not different. Exposed fish swam 40% faster on average, however, they spent more time encamped—a behavior where fish are active but in a repetitive and small space— than exploring or transiting, hence this difference in speed was not reflected in their use of space. Exposed fish in fact had a more constrained and aggregated distribution in the landscape than control fish, associated with a preference for areas with the presence of macrophytes. Our results support previous findings on the increase in swimming activity of fish exposed to oxazepam. Moreover, our approach incorporating landscape use and resource selection provides a more comprehensive picture of the full extent of pharmaceutical effects on fish behavior. Our findings highlight the importance of considering ecological context in behavioral ecotoxicology studies to better understand the risks associated with pharmaceutical contamination at population and ecosystem levels.

### **3.20.P-Th381 From Science to Practice: Ecotoxicological Insights in Malaria Vector Control in Burkina Faso**

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In the ongoing battle against malaria in Sub-Saharan Africa, innovative and contextually appropriate vector control methods are crucial. Our study in Burkina Faso explores a novel vector control strategy with the endectocide ivermectin to control *Anopheles* mosquitoes. Using ivermectin-treated cattle, we also integrate the One Health mindset that interlinks human, animal, and environmental health. This strategy, a part of our ANIVERMATE project, aims not just for scientific efficacy but also on its alignment with local agricultural and environmental practices.

Central to our research is the utilization of long-acting ivermectin formulations in cattle, offering a dual benefit of sustained mosquito control and enhanced animal health. However, ecotoxicological guidance of this strategy, particularly considering the well-documented environmental impact of ivermectin, is pivotal. Our work includes proposed risk mitigation measures, such as the use of "fosses fumières" – traditional manure storage pits. These pits are crucial in the local farming cycle and represent a potential site for environmental interaction with ivermectin residues. To understand how dung of treated cattle would be used under field conditions, we also surveyed farmers from three adjacent villages on their use of dung (as a fertilizer) and regarding their agricultural routines.

Our findings emphasize the necessity of aligning scientific innovations with the realities of local farming practices. We delve into the dynamics of ivermectin excretion and degradation in cattle dung and explore tailored risk mitigation measures that aim to protect the unique environmental conditions of West African agroecosystems.

Our research, supported by a robust modeling dataset, demonstrates the potential of ivermectin-treated cattle for malaria vector control. Bridging advanced vector management with ecotoxicological awareness, our study aligns with the One Health concept, emphasizing an integrated approach for ecosystem health, animal welfare, and community well-being. This method not only targets effective malaria control but also contributes to sustainable health practices in Sub-Saharan Africa.

### **3.20.P-Th382 Bioaccumulation of pharmaceuticals and metabolome investigation in trout – from egg to young-of-the-year fish**

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The emissions of micropollutants from wastewater treatment plants (WWTPs) into the receiving surface water is a recognised threat to the aquatic environment. The adverse effects of released chemicals on aquatic organisms belong to the most accented ones. In this study, brown trout (*Salmo trutta*) eggs were deployed to a natural stream, where treated wastewater contributed up to 25% of the total stream flow. The eggs placed upstream of the WWTP served as a reference locality. The bioaccumulation of selected pharmaceuticals and their metabolites in three developmental stages of brown trout (fish egg, yolk sac fry and young-of-the-year fish) was studied using liquid chromatography with high-resolution mass spectrometry. Besides the bioaccumulation, the changes in metabolome and exposome were evaluated. Fifteen pharmaceuticals were found with clear bioaccumulation trends with time since hatching. The highest concentrations were observed for antidepressants sertraline and its metabolite norsesertraline, trazodone and for cardiovascular drug telmisartan. It was proven that individual developmental stages bioaccumulated different pharmaceuticals with different intensities. Based on the non-targeted screening, significantly changed signals (up- and downregulated) increased with exposure time. Some endogenous and exogenous compounds in exposed fish differ from fish from the reference locality. The treated wastewater caused bioaccumulation and has a negative impact on the metabolome in the early developmental stages of brown trout. The study was conducted under project No. 20-04676X (Czech Science Foundation).

### 3.21.A Polymers and Their Chemicals: Environmental Fate, Hazards, and Risk Assessment

#### 3.21.A.T-01 A Draft Framework for Risk-Based Prioritization & Evaluation of Additives & Polymer-associated Chemistries (APAC)

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Widespread global use of polymers and plastic has led to increased stakeholder interest in the health & environmental assessments of these materials. Formal frameworks (i.e., RISK21, Canadian ERC) may require specific data types, developed with prescribed methods, and largely focused on non-polymeric substances. These data are not always available and can result in inaction, or conservative decision making. This is due to the propensity for regulatory or policy decisions to rely primarily on hazard information, without comprehensively considering exposure information. Assessing risk and making risk-based decisions requires deliberate consideration of a complex, and often dynamic, suite of information. For risk assessments that are highly challenging (e.g., large numbers of substances), approaches which screen and prioritize data inputs is a practical methodology for parsing out and addressing complexity in a stepwise approach. This framework for screening and prioritizing additives and polymer-associated chemistries (APAC) was developed to parameterize the large, diverse set of chemistries that are utilized in the manufacture and processing of polymers by considering relative chemical hazards and potential polymer-related exposure profiles together to form the initial basis for a practical and informed risk assessment. The framework leverages existing datasets, mines global databases, and utilizes predictive modeling methodology in a tiered approach to move toward the future goal of fit-for-purpose polymers risk assessments, and is intended to be an accessible approach for all stakeholders with interest in polymers risk assessment. To evaluate the utility of the framework for a large, diverse dataset, a preliminary analysis utilizing the UN database was performed. Substances were assigned to hazard and exposure tiers (based on the nature of available data for each substance) and a matrix was developed to visualize the level of quantitative risk assessment which could be performed for the database. It is estimated that at a minimum Tier 1 hazard and exposure assessments may be conducted for ~ 83.8% of substances from information identified or which may be available. For the remaining substances, risk-based screening may be possible with additional data sources and/or further manual curation.

#### 3.21.A.T-02 The Role of UV Light on the Aquatic Leaching and Transformation of Plastic Additives

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Functional additives enhance the performance, functionality and stability of plastic products. When released, these additives can, however, become of significant environmental concern. For example, floating plastic can pose a threat to aquatic environments through leaching of chemicals. Solar radiation is expected to affect the leaching of such floating material; the ongoing processes are complex and currently poorly understood. In this work, eight different plastic consumer products were leached under UV radiation under controlled laboratory conditions simulating eight months of environmental weathering to study the release of additives and the formation of possible photo-transformation products. A broad analytical toolbox was employed covering metal(loid), microplastic, target and non-target analysis to characterize the leachates comparing UV-treated and dark control samples. Environmental concerns were estimated using different toxicity tests based on e.g. algae, snails and human cell-lines. The leachates were found to exhibit a multi-stressor scenario by containing small plastic particles, diverse elements and organic additives. Some leachates were found to pose toxic concerns. However, the observed effects could not easily be linked to the analytical data due to possible analytical blind spots and its complexity. More detailed investigations into possible transformation products using high-resolution mass spectrometry revealed the presence of several tentatively identified structures that could explain parts of the anticipated analytical gaps. This research highlights the need for a broad analytical toolbox to study the role of solar radiation in already complex natural leaching processes and concludes that cross-discipline research projects are crucial to the understanding of threats posed by environmental plastic pollution.

#### 3.21.A.T-03 Exploring Past and Present Inputs of Organophosphate Ester Plastic Additives in the French Atlantic Coast by Sediment Core Analysis

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Organophosphate esters (OPEs) flame retardants and plasticizers present multiple environmental sources, exhibit a number of hazardous effects and are among the most abundant organic plastic additives found in the marine environment. Due to their generally high octanol-water partition coefficients, most OPEs have the tendency to bind to suspended particulate matter, accumulating in sediment over time. The study of their vertical profiles in dated sediment cores allows the retrospective investigation of their deposition and exposure history. However very few investigations reported on the vertical distribution of OPEs in marine sediment cores. In this work we (1) investigated the occurrence of parent OPEs (tri-OPEs) and their major

degradation metabolites (di-OPEs) in horizons covering the last 50-100 years from three sediment cores collected in the Gulf of Biscay (North-East Atlantic Ocean) and, (2) explored the vertical profiles of these contaminants to assess historical and present concentrations and exposure trends in the study area. Sediments cores were collected on board of the R/V Thalia (IFREMER) in May 2022 (campaign ROCCHSED2022, DOI: 10.17600/18002069.) in three sites subject to different anthropogenic pressures by using an interface corer equipped with stainless steel tubes. Once in the lab, the cores were sliced into 1 cm horizons and dated. OPEs were extracted by sonication, extracts cleaned-up by solid phase extraction and quantified by isotopic dilution LC-ESI-MS/MS. An increasing north-to-south OPE loading trend was observed, with core 22-04 (southernmost site) exhibiting the highest median concentrations of 2.3 and 0.4 ng g<sup>-1</sup> dw for  $\Sigma$ tri-OPEs and  $\Sigma$ di-OPE, respectively. This core showed also the highest detection frequency (50-78%). Our results show that OPEs seem to be present in the area already in the early 50's, although only few compounds (mostly alkyl-OPEs) were detected at low concentrations (pg g<sup>-1</sup> dw level). Although some general trends were observed for  $\Sigma$ tri-OPEs in the core 22-04, a high concentration variability was observed over time, pointing to heterogeneous input patterns. Vertical profiles of individual tri- and di-OPEs are under evaluation for this sediment core as well as the other two sites, and may help better assessing the past and present inputs of OPEs in the area. Relative distribution of individual OPEs along the vertical profiles and tri-/di-OPE ratios will be also discussed in the presentation.

### 3.21.A.T-04 Current Levels of Microplastic Pollution Impact Wild Seabird Gut Microbiomes

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Given the large amounts of microplastics in the environment, their long-lasting nature, and the countless wildlife species exposed to microplastic pollution throughout their life histories, it is imperative to understand how wildlife health is impacted by microplastics in their current environmental concentrations, which are only projected to rise in time. Despite its vital role in host health and its propensity to change in taxonomic and functional diversity and composition in animals subjected to anthropogenic and environmental stressors, the gut microbiome represents a new avenue in microplastics research that has not been thoroughly explored as a possible impact point for microplastic pollution. Here, we studied the gut microbial response in two different parts of the gastrointestinal tract to varying degrees of microplastic ingestion in two different seabird species known from long-term monitoring to ingest (micro)plastics: the northern fulmar, *Fulmarus glacialis* ( $n = 27$ ; a bioindicator for plastic pollution) and Cory's shearwater, *Calonectris borealis* ( $n = 58$ ). This study is the first to describe impacts of environmentally relevant concentrations of microplastics ingested by wildlife species on the diversity and composition of the gut microbiome. Using this multi-species study system, we found that effects of microplastics on gut microbial composition are species-specific, whilst effects on gut microbial diversity are shared between the two species. However, regardless of host species, our results show a decrease in commensals and increase in potentially pathogenic, antibiotic resistant, and plastic-degrading microbes associated with microplastic ingestion at concentrations already present in the environment. Since humans also ingest plastic particles (besides stool, microplastics have even been found in human placentas, blood and lungs), this study puts into question the potential effects microplastics may have on human microbiomes and thus human health.

### 3.21.A.T-05 Effects of Di-Butyl Phthalate and Di-Ethyl-Hexyl Phthalate at Environmental Doses on Health and Development of *Bombus terrestris* Microcolonies

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The urban environment can have a contrasted role on wild pollinator population settlement. It acts both as a refuge with high floral diversity and zero-phyto policies in Northern cities, but also presents severe constraints such as soil impermeabilization, heat islands and pollution. With the recent legislations implemented in Northern cities to reduce fossil fuel exhaust, the atmospheric pollution profile has changed, revealing organic volatile compounds from petrochemical industry, such as phthalates as main air pollutants. These molecules have endocrine disrupting effects on vertebrate models but have been less studied in invertebrates and especially in terrestrial invertebrates. In the context of the current insect decline crisis, it seems important to understand the impact of such ubiquitous molecules on their health and development as they can disrupt crucial hormonal regulations such as the ecdysteroid pathway. In this study, we exposed *Bombus terrestris* workers in microcolonies to Di-Butyl Phthalate (DnBP) and Di-Ethyl-Hexyl Phthalate (DEHP) alone and in mixture at environmental doses, found in and nearby the city of Lille, France. We investigated the effects of such exposure on microcolony health, worker mortality and larval development. Preliminary results show an increased in worker mortality caused by repeated exposure to DEHP alone and in the mixture, associated with a decrease in adult male production in microcolonies exposed to the mixture. Moreover, repeated exposure to DnBP affected worker lipid content, with increased mass in late stage larvae, suggesting transgenerational obesogenic effects of this molecule. Phthalate exposure seems thus to affect both adults and larvae of *B. terrestris*. It seems therefore important to understand the impact of those molecules on the health of insect populations and especially on bees which population trends show a severe decline since the past seventy years.

### 3.21.B Polymers and Their Chemicals: Environmental Fate, Hazards, and Risk Assessment



### 3.21.B.T-01 Recycled Polyethylene: A Closer Look at Chemical Complexity and Environmental Consequences

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Plastics, ubiquitous in our environment, present a significant challenge despite efforts to reduce their impact. Plastic pollution remains a pressing threat, with regulatory gaps and >13,000 chemicals used in production. Recycling of plastics is fraught with transparency issues, further contributing to environmental risks, particularly in low-income nations burdened by plastic waste from high-income countries. Mechanical recycling facilities emerge as sources of microplastic pollution, releasing toxic compounds into the environment. To address these challenges, we collected recycled plastic pellets from Global South recycling facilities, analysing them for potential toxicity and identifying substances requiring regulation. The study advocates for transparency and reduced chemical use in the plastics industry.

Materials and methods involve collecting HDPE samples globally, subjecting them to chemical analysis using LC-HRMS. The study employs a robust framework, including UAE and bioassays on *Raphidocelis subcapitata*, assessing the impact of plastic extracts on algal growth. Results reveal diverse contaminants in recycled plastics, with N-Ethyl-o-toluenesulfonamide and N,N-dimethyl-p-phenylenediamine as notable compounds. Pesticides, pharmaceuticals, industrial compounds, among others were detected through Targeted and Non-Targeted Screening analysis. The chemical trojan horse phenomenon suggests plastics could release absorbed chemicals into the environment, threatening biodiversity. Bioassays show growth inhibition and enhancement effects on microalgae, indicating potential environmental issues.

In conclusion, the study underscores the challenge of diverse chemical adsorption in recycled plastics and the complexity of mixture toxicity, making the identification of specific substances challenging. Suboptimal production practices enable potentially harmful trace substances to accompany consumer products, emphasizing the need for improved waste management and sustainable plastic practices.

### 3.21.B.T-02 Effect-Directed Analysis of Endocrine Disruptors in Plastic Food Packaging

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Plastics are chemically highly complex materials with more than 13,000 substances either used in the manufacturing of plastics (e.g., plasticizers, antioxidants) or non-intentionally added during production processes (e.g., reaction byproducts, impurities). Despite established evidence linking certain plastic compounds (e.g., bisphenols and phthalates) to endocrine disruption, the specific toxic compounds leaching from everyday plastic products remain largely unknown. Therefore, our study aims at identifying the endocrine compounds leaching from plastic food contact articles (FCAs) through an effect directed analysis. Four plastic products made of polystyrene (PS), polyvinylchloride (PVC) and polyurethane (PUR) were leached into a water-ethanol mixture for 10 days at 40°C according to European Regulation 10/2011/EU. Employing high-performance liquid chromatography, we separated the resulting plastic leachates into 60 fractions. Reporter gene assays for nuclear receptors relevant to human health, including peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ), estrogen receptors alpha (ER $\alpha$ ), and androgen receptor (AR), were used for analysis. For the identification of the causative chemicals, the active fractions were analyzed by non-target high-resolution mass spectrometry. For the PVC drinking tube, a single estrogenic fraction, constituting for 56% of the activity of the original leachate, was identified. This suggests that most of the estrogenic activity is induced by chemicals contained in one fraction eluting with 100% methanol. The two PUR hydration bladders exhibited three anti-androgenic fractions, each accounting for less than 10% of the original samples' activity. Ongoing efforts are focused on identifying chemical features within the active fractions. This research underscores that plastic FCAs leach endocrine disrupting chemicals into food simulants and by identifying the causative compounds, can enable monitoring the human exposure and better understanding downstream effects.

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### 3.21.B.T-03 Translating the hazards of additives in plastic mulch films to the risk of release

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Plastic mulch film supports improved food security, particularly in regions of marginal productivity. Associated with these films are organic and metal additives, which are not chemically bound, and thus susceptible to loss to the wider soil environment prior to polymer breakdown. There are known hazards associated with heavy metals and some additives (e.g., phthalates, organophosphites) included in plastics, yet the overall additive and metal content still remains relatively unknown,

as does the potential risk of these components leaching to the wider environment. In this study, we analysed LDPE (n=47) and biodegradable (n=5) plastic mulch films from China for solvent-extractable additive content via GC-MS and metal content via TXRF. The mulches were then leached to explore the potential extent and composition of the chemical burden arising from organic compounds and metals. Lubricants (fatty acids, amides, alcohols) and antioxidants (Irgafos® 168, Irganox® 1076) were the most abundant additives. Plasticisers, both phthalate and non-phthalate, were present at lower abundances and, particularly for biodegradable films, were dominated by additive and polymer degradation products. The overall metal content was higher in biodegradable (7.9±0.7% w/w) than LDPE (0.88±0.17% w/w), dominated by Ca, Na and Al with only minor contributions from heavy metals (Fe, Zn, Ti, Cd). The average leached content from LDPE was 71.0±9.9 mg kg<sup>-1</sup> (equiv. of 2.3% extractable from the new plastic) and 3024±146 mg kg<sup>-1</sup> for biodegradable plastic (equiv. of 28% extractable from the new plastic). A total of 612 unique compounds were present in the leachate, yet >50 were present in the new films. The majority of the leachate content (80%) has not undergone any regulatory scrutiny, and thus are of unknown concern following release. As the contribution to the total metal content of heavy metals (i.e. high concern) was low, leaching of these metals into both acid and water was also low compared to other metals (7.7±0.7 g ha<sup>-1</sup> and 27.5±10.5 g ha<sup>-1</sup>, for LDPE and biodegradable mulches, respectively). Biodegradable mulches had higher leaching of both organic and metal additives, raising the question of pollution swapping if used as an alternative to LDPE mulch. This work revealed the complexity of the chemical burden posed by both LDPE and biodegradable mulch films, which is not fully reflected by the composition of the parent mulch and must be considered in future ecotoxicology assessments.

### 3.21.B.T-04 Occurrence of novel polymer additives in house dust and its association with allergic diseases

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Polymer additives are used to improve strength, durability, flexibility, and many other properties of materials and that account for more than 10,000 different chemicals currently used. Human exposure to such chemicals can occur through various pathways, and the study of environmental exposures have generated different causal links to adverse health consequences. Our work used house dust samples (n=191) from the Dustsafe Aotearoa project, New Zealand, together with household participants demographic and self-reported clinical conditions. Twenty-four house dust contaminant concentrations were determined as well as house dust extract (HDE) *in-vitro* nuclear factor kappa B (NFκB) activity. Out of 24 monitored chemicals, five bisphenol analogues (BP) (BPE, BPP, BPM, BPC, and BPG) were not detected in any of the dust samples. Six chemicals were detected in less than one third of the samples (atrazine, BPBP, BPAP, BPAF, BPTMC, and BPPH). Of the BPA and analogues groups, only Bisphenol S, Bisphenol A and Bisphenol BP were frequently detected (detection frequency of 99%, 60.6% and 35.2%, respectively). 1,3-diphenylenimine (DPG) showed the second highest median concentration in house dust of 735 ng/g (95% CI=598 ng/g - 831 ng/g) and a higher detection frequency of 99%. In this work, we report for the first time on the association of occurrence of the polymer additive 1,3-diphenylguanidine (DPG) in house dust and atopic dermatitis. Households with atopic dermatitis participants (n=58) had a median DPG concentration of 809 ng/g (95% CI=700 ng/g - 1,050 ng/g) versus 392 ng/g (95% CI=316 ng/g - 447 ng/g) of those of non-atopic dermatitis households (n=133). House dust extracts from homes with atopic dermatitis participants also showed a greater activation of NFκB response with bioactivity having a strong relationship with DPG concentrations. To verify the association of DPG with the observed induction of NFκB pathway, an effect-directed analysis of a pooled house dust extract was performed, and DPG-containing fractions were found to be responsible for 11.2% of the total observed NFκB activity, the major contributing hydrophilic fractions. While DPG is recognized as an allergen, to the best of our knowledge, this is the first report that associates its occurrence as an environmental contaminant with atopic dermatitis incidence, suggesting a potential risk from human exposure to house dust containing high concentrations of DPG.

### 3.21.B.T-05 Hazard Assessment of 6PPD-quinone across fishes of commercial, cultural, and ecological importance

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Stormwater runoff from urban landscapes has recently been linked to mass mortalities of coho salmon in the U.S., also dubbed urban runoff mortality syndrome (UMRS). The chemical responsible has been identified as N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q), a transformation product of the rubber tire antioxidant 6PPD. This presentation summarizes a series of studies that assessed the acute toxicity of 6PPD-quinone across eight other fishes of commercial, cultural, and ecological importance in North America, and to characterize the specific mechanisms that drive toxicity using acute and sub-chronic experiments with select fishes. Acute toxicity differed greatly among species: No mortality occurred for Arctic char (AC), brown trout, bull trout, westslope cutthroat trout, and white sturgeon even at the highest measured concentrations (>13 ug/L), while lake (LT), brook, and rainbow (RBT) trout were sensitivity to 6PPD-Q exposure with LC50 values between 0.33 and 0.59 ug/L. Furthermore, sub-chronic exposures of RBT and LT demonstrated teratogenic effects in

both larval RBT and LT. Experiments with primary cultures of RBT gill cells suggest that the mechanism of toxicity may be related to uncoupling the mitochondrial electron transport chain. Comparative cardiac ultrasound, electrocardiography and blood gas analysis revealed significant decreases in hemoglobin oxygenation and sympathetic stimulation in sensitive RBT but not insensitive AC, further supporting this hypothesis. Whole transcriptome analysis in RBT identified several molecular toxicity pathways that may explain the apical effects described above. Further research supporting development of a comprehensive toxicity pathway model supporting risk assessment of 6PPD-quinone across fishes is underway.

### 3.21.P Polymers and Their Chemicals: Environmental Fate, Hazards, and Risk Assessment

#### 3.21.P-We206 Predicting the Environmental Implications of Tire Compounds: Property Estimation and Environmental Modelling

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Tires, as an integral part of modern transportation, undergo constant wear and tear, releasing a complex mixture of tire-derived chemicals (TDCs) into the environment. This release process contributes to the dispersion of TDCs across various environmental compartments, including soil, water bodies, and air, as recent monitoring work has demonstrated. However, the environmental distribution, transport mechanisms, and transformation processes of these compounds are not well understood. This is partly due to insufficient information on their physico-chemical properties, which are crucial for understanding their environmental behaviour. Furthermore, the full range of chemicals released from tire pollution is still unknown, adding complexity to environmental impact assessments. This project seeks to address these research gaps by first estimating the missing physico-chemical properties of TDCs. To do so, a list of >200 compounds known to be used in synthetic rubber has been extracted from the PubChem database. To enhance the scope of this work, we conducted non-target analysis (NTA) on tire-wear particle (TWP) leachate extracts to identify additional TDCs, thus broadening our dataset for more comprehensive property prediction and fate modelling. NTA was conducted on both non-polar and polar TWP extracts using two-dimensional gas chromatography-mass spectrometry (GCxGC-ToF-MS) as well as liquid chromatography-high resolution mass spectrometry (Orbitrap LC-HRMS). To estimate the physico-chemical properties for each chemical, this project leveraged prediction software tools such as the Estimation Programs Interface (EPI) Suite, OPEn structure–activity/property Relationship App (OPERA), and BIOVA COSMOtherm. The partitioning tendencies of the TDCs were evaluated through interpretive chemical space plots. Environmental effect metrics, such as the fish bioconcentration factor and the acute oral systemic toxicity in rats were also predicted. Subsequently, this work applied level III steady-state fugacity models as environmental fate assessment tools to better understand the distribution, transformation, and persistence of these contaminants in different environmental matrices. This work will allow for the prioritization of these compounds in future environmental monitoring campaigns and empirical toxicity assessments as well as guide the refining of their chemical management strategies.

#### 3.21.P-We207 Plasticizers and flame retardants in rubber recycling and recreational surfaces

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Granulated vulcanised elastomeric materials as crumb rubber sourced from used tyres present both a serious environmental challenge and new technological opportunities. These materials are chemically complex and therefore difficult to recycle, and also can contain toxins and are present in huge quantities. This work will focus in used-tyres used as turn infill and as base layer for playgrounds, mainly made of styrene-butadiene rubber (SBR), as well as ethylene propylene diene monomer (EPDM) that is used as wear layer in playgrounds. The aim of the study is to assess the occurrence of organophosphate esters (OPE) and phthalates in these materials, as these compounds are used as plasticizers and flame retardants, and some of them have been demonstrated to present a risk for human health.

In the first part of the study, OPEs were analysed in different EPDM and SBR samples provided by Barcelona City Council, being the same materials used in the playgrounds. Additional experiments are being carried out to look for degradation by the daylight during a 3-month period were carried out, with the analysis of OPEs and also of phthalates and alternative compounds to phthalates.

Fifteen out of the 20 analysed OPEs were detected in EPDM and SBR samples, with concentrations ranging between 71.2 and 2460 ng/g for the EPDM and SBR samples used in playgrounds (red, green, yellow, blue and black), and between 8.89 and 118 µg/g in SBR used in sport, showing a huge difference between both kind of samples.

It was found that for most cases OPE concentration increased after crushing the sample, as extraction efficiency is also increased. That was an exception for SBR samples used in sport fields, as those ones were coated with painting. That resulted into a reduction of concentration after crushing the sample, as the amount of coating was diluted, giving a more realistic result about the bulk concentration.

In samples from playgrounds, there were five dominant OPEs: TEP, TCEP, TPHP, TNBP and TCIPP, while in the case of sport samples the TEHP was the dominant congener. It is crucial to note that TEP, TPHP, and TCIPP present non-carcinogenic

risks, while TCEP, TNBP, and TEHP pose both carcinogenic and non-carcinogenic risks. This underscores the need for additional studies to assess whether the use of these materials in recreational surfaces could indeed pose health risks.

### **3.21.P-We208 Exploring Toxicity of Tire Particles and Tire-Related Chemicals with Bioassays on High-Performance Thin-Layer Chromatography Plates**

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Tire and road wear particles (TRWP) contain complex mixtures of chemicals and release them to the environment, and potential toxic effects of these chemicals still need to be characterized. We used a currently available surrogate for TRWP, cryogenically milled tire tread (CMTT), to isolate and evaluate effects of tire-associated chemicals. We examined organic chemical mixtures extracted and leached from CMTT for the toxicity endpoints genotoxicity, estrogenicity, and inhibition of bacterial luminescence. The bioassays were performed after chromatographic separation on high-performance thin-layer chromatography (HPTLC) plates. CMTT extracts were active in all three HPTLC-bioassays with two estrogenic zones, four genotoxic zones, and two zones inhibiting bacterial luminescence. Two types of aqueous leachates of CMTT, simulating either digestion by fish or contact with sediment and water, contained estrogenic chemicals and inhibitors of bacterial luminescence with similar profiles to those of CMTT extracts. Of eleven tested tire-associated chemicals, two were estrogenic, three were genotoxic, and several inhibited bacterial luminescence. 1,3-Diphenylguanidine (DPG), transformation products of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), and benzothiazoles were especially implicated through comparison to HPTLC retention factors in the CMTT samples. Other bioactive bands in CMTT samples did not correspond to any tested individual chemicals. Tire particles contain and can leach complex mixtures of chemicals to the environment. While some known chemicals contribute to estrogenic, genotoxic, or antibacterial hazards, unidentified toxic chemicals are still present and deserve further investigation. In summary, HPTLC showed that few chemicals were responsible for the detected effects and implicated known chemicals as likely causative toxicants. Our work expands the understanding of potential adverse effects from tire particles and helps improve the link between those effects and the responsible chemicals.

### **3.21.P-We209 Monitoring and Modelling of Phenylenediamine Antioxidants and Their Transformation Products in Urban Snow – An Important Medium Linking Tire Additive Emissions and Aquatic Loadings**

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Chemical emissions from tires are an emerging environmental concern. They can have toxic or harmful effects on organisms in aquatic ecosystems. Snow acts as a natural filter, trapping and removing various pollutants from the atmosphere, including chemicals from traffic emissions. However, as snow melts and runoff occurs, these chemicals can be released back into the environment, potentially contaminating aquatic environment. To understand the role of snow in trapping chemicals additives in tires, a monitoring study was conducted to characterize concentrations of chemical trapped in road site snow and developed model simulations to characterize chemical processes in snow. The snow model was incorporated into the Multimedia Urban Model to study the implication of snow melt on the chemicals' mass flow within urban environment. The study found that the concentrations of tire additives in snow were significantly influenced by traffic levels. The highest concentrations were found in snow from high traffic roads. The study also identified a number of transformation products of tire additives in snow, which suggests that these chemicals can undergo chemical changes in the environment. The study's findings highlight the importance of understanding the fate and transport of tire additives in snow, particularly in northern cities where snow plays a major role in the urban water cycle.

### **3.21.P-We210 Green Analysis of Additives from Plastic Samples by In-Tube Extraction Dynamic Headspace Coupled to GC-MS/MS**

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The analysis of plastic additives is necessary to evaluate the real risk (for the environment and the human health) posed by the presence of plastics in the environment. The European Chemical Association (ECHA) characterized over 400 substances used as plastic additives. Some additives like bisphenol A (BPA), Tinuvin328, Tinuvin 327, and many phthalates have been identified by ECHA as substances of very high concern (SVHC).

In this work, In-tube extraction dynamic headspace sampling (ITEX-DHS) combined with gas chromatography (GC) and mass spectrometry (MS/MS) method was optimized for the analysis of about 50 plastic additives from plastic samples. Multivariate and univariate approaches were used to optimize the many parameters affecting the extraction and desorption efficiency. Quantitation of target analytes was performed by matrix matched calibration, using isotopically labelled standards as internal standards.

Good analytical performance characteristics were obtained: trueness was between 70-128%, the intermediate precision between 3-24%, with good sensitivity (quantitation limits of the method ranging from 0.2 ng g<sup>-1</sup> (DnOP) to 0.37 µg g<sup>-1</sup> (Ionox

100)). The greenness of the optimized method was calculated using AGREE software, obtaining an overall score of 0.82 (in a range from 0 for the less green, and 1 for the greenness) due mainly to be a solvent-less and fully automatic method.

This is the first approach using ITEX-DHS for the quantitative analysis of about additives from plastics, and supposes a good alternative for the reliable quantitative analysis and monitoring of additives from plastics and microplastics.

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#### 3.21.P-We211 Human and Environmental Exposure by Synthetic Antioxidants: Insights from Wastewater

Corina Meyer<sup>1</sup>, Mara Lisa Baer<sup>2</sup> and Juliane Hollender<sup>3</sup>, (1)Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland, (2)Institute of Biogeochemistry and Pollutant Dynamics, ETH Zurich, Switzerland, (3)Environmental Chemistry, Environmental Chemistry, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Switzerland Synthetic antioxidants (SAOs) are of widespread use in everyday life products, including polymeric materials, medicinal face masks, personal care products and food. They act by preventing oxidative reactions and extending product shelf life. As antioxidants are additives and not chemically bound to their substrates, leaching from materials and detection in wastewater treatment plants (WWTPs) has been reported. Correspondingly, uptake by humans is expected through contact to materials. However, little is known about the human and environmental exposure of antioxidants. Therefore, this study aims (i) to identify SAO metabolites in untreated wastewater to get some insight into human exposure and (ii) to analyze the abatement behavior of the parent compounds and their metabolites through the different treatment steps in WWTPs to understand the environmental exposure. A MS/MS library of SAO metabolites was generated by human liver S9 incubation experiments, combined with online-SPE-LC-HRMS/MS analysis. This library, containing about 1000 potential metabolites, was compared to influent samples of three Swiss WWTPs, leading to approximately 50 matches. Using machine learning and *in silico* fragmentation tools, structure elucidation was so far possible for one compound (fenoazan), which was confirmed by a reference standard. This result gives first hints with regard to human exposure towards SAOs. To further elucidate exposure pathways, medicinal face masks were analyzed by the same analytical method. Their extracts contained over 30 different antioxidants. Total SAO concentrations of around 100 µg per mask were found. Current analyses examine the wearing of face masks as uptake pathway. Besides the detection of metabolites in untreated wastewater, knowledge about the abatement of parent SAOs through the different treatment steps in WWTPs is missing. Thus, the same three WWTPs, equipped with an advanced treatment step (ozonation or granular activated carbon filtration), were sampled and analyzed by online-SPE-LC-HRMS/MS after every treatment step to assess environmental exposure by WWTPs. Results indicate incomplete abatement of some SAOs. Overall, humans and the environment seem to be exposed to synthetic antioxidants and further research is required to understand the uptake of SAOs into the human body, their metabolism and their behavior in wastewater treatment.

#### 3.21.P-We212 Halogenated Flame Retardants in Dust collected from Indoor Environments: Homes, Offices, Kindergartens and High Schools

Adrián de la Torre<sup>1</sup>, Irene Navarro<sup>2</sup> and María Ángeles Martínez<sup>2</sup>, (1)Center for Energy, Environmental and Technological Research (CIEMAT), Madrid, Spain, (2)Center for Energy, Environmental and Technological Research (CIEMAT), Spain In March 2023, the European Chemicals Agency (ECHA), released its Regulatory Strategy for Flame Retardants and identified aromatic brominated flame retardants as candidates for an EU-wide restriction. In this report, a high exposure potential to consumers during article service life was associated with the use of items such as textiles, household furniture, electronic equipment or even toys through skin contact or indoor dust ingestion. To address this issue the present study investigates the occurrence of 51 flame retardants (FR) including polybrominated diphenyl ethers (PBDEs), novel brominated flame retardants (DBDPE, BTBPE, HBBZ, PBEB) and dechloranes (Clordane Plus, Dec 602, 603, 604 and Declorane Plus (DP)) in indoor dust samples collected from homes, offices, kindergarten and high schools from Spain. The validated analytical method (SRM2585) including extraction (hexane:acetone; 3:1 v/v), fractionation (florisil column), purification (acid silica column), HRGC-MS/MS (Agilent 8890-7010c) analysis and isotope dilution for quantification, was applied to 85 settled (<0.5 m) and suspended (>0.5 m) dust samples.

Brominated targeted analytes presented high quantification frequencies (Qf) in all indoor environments, with a pollutant pattern dominated by  $\sum_{42}$ PBDEs (445 ng/g, 100%; median, Qf) followed by DBDPE (244 ng/g, 100%), BTBPE (6.77 ng/g, 40%) and HBBZ (2.47 ng/g, 51%). However, for dechloranes, only DP was quantified in 81% of the samples (10.2 ng/g). Interestingly, no differences ( $p > 0.05$  Mann-Whitney test) were obtained between suspended and settled dust in terms of FR content. Positive correlations ( $r_s > 0.4$ ,  $p < 0.01$ ; Spearman test) were found between BDE-209, DBDPE and DP suggesting that BDE-209 substitution is taking place, but also reflecting similar application areas, and/or behavior once sloughed off from consumer products. Important differences were found between indoor environments. Dust obtained from homes and offices presented higher PBDEs and DBDPE ( $p < 0.05$ ; Kruskal-Wallis test) concentrations compared to samples collected from

kindergartens and high schools. Finally, data obtained were used to determine estimated daily intakes (EDI) via dust ingestion and dermal absorption for toddlers and adults at central and upper percentiles. Calculated EDI levels even at worst case scenario were below available reference dose (RfD) values in all cases.

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### **3.21.P-We213 In-Vehicle Exposure of Southern California Commuters to Tris(1,3-dichloro-2-propyl) phosphate** *David C. Volz<sup>1</sup>, Aalekhya Reddam<sup>1</sup>, Nicholas Herkert<sup>2</sup> and Heather M. Stapleton<sup>2</sup>, (1)Department of Environmental Sciences, University of California Riverside (UC Riverside), (2)Nicholas School of the Environment, Duke University*

Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) is an additive, organophosphate-based flame retardant (OPFR) introduced within vehicle parts to meet a stringent, federal-level flammability standard within the United States. As TDCIPP is a semi-volatile organic compound, TDCIPP migrates from end-use products, resulting in human exposure to TDCIPP-contaminated air and/or dust within the interior of vehicles. To our knowledge, human OPFR exposure as a function of total commute time had not been evaluated prior to 2019. Therefore, the overall objective of our first, one-week study in 2019 was to leverage silicone wristbands to monitor personal OPFR exposure within a subset of commuter vs. non-commuter undergraduate students (N=88) at the University of California, Riverside. Out of 22 OPFRs analyzed, 13 OPFRs were detected on at least 62 wristbands (70% detection rate). Among these 13 OPFRs, TDCIPP was the only OPFR that was significantly predicted by total commute score, suggesting that longer commutes were associated with increased human exposure to TDCIPP. Therefore, the overall objective of our second, two-week study in 2020 was to determine if a decrease in interior car dust resulted in mitigation of personal TDCIPP exposure for participants (N=49) who spend a significant amount of time in their personal vehicles. Once participants enrolled in the study, participants were distributed across four groups that determined when the participants wiped their car interiors during the two-week study. Interestingly, there was no significant difference in wristband TDCIPP concentrations among the different wiping groups despite concentrations being higher than those of non-commuters. Moreover, when all of the no-wipe and wipe data were pooled to enhance statistical power, we detected no significance difference in TDCIPP concentrations between these two groups. Therefore, findings from our 2020 study suggest that the correlation between TDCIPP exposure and longer commutes within our 2019 study may have been attributed to direct partitioning from vehicle parts into wristbands via car interior air. Given that Americans within densely populated regions across the United States spend one or more hours commuting on a near-daily basis, there is a critical need to understand the potential human health implications of chronic TDCIPP exposure within vehicles, particularly within traffic-congested areas such as Southern California.

### **3.21.P-We214 Time Trends of Flame Retardant Additives in Cars**

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Vehicles are a unique indoor environment as they are full of synthetic materials, textiles and electronics, many of which contain a complex mixture of additive chemicals to impart safety and appearance properties. In addition, vehicles are subject to significant external impacts through variations in temperature and radiation that can influence the emission, degradation and fate of plastic additives.

We investigate the presence of brominated and organophosphate flame retardant (FR) additives in vehicles and evaluate the influence of vehicle age on the profile and levels of FRs detected. Vehicle dust was used as a surrogate matrix to indicate FR use in vehicle parts. Ten individual vehicles of two models of a Czech brand were tested. The two models were selected because vehicle assembly occurs at European sites, suggesting compliance with EU chemical regulations. The model years spanned 1996 to 2021.

We expected a shift in the composition of FRs over the 25 year span of vehicles, yet this was not broadly supported by the dust concentrations. Distinct differences were only seen in the levels of polybrominated diphenyl ether (PBDE) congeners associated with the penta-BDE commercial mixture. In the oldest car from 1996, the concentration of BDE 47 was ~1000x higher than in all subsequent vehicles, attributed to past use of penta-BDE in vehicle foams. However, no time trends were observed for other FRs. BDE 209 was measured at levels of 97.2-39201 ng/g in car dust (median 3260 ng/g), with the highest value found in a car from 2009, and no significant differences according to car age, nor any evidence of a transition to alternative FRs. The elevated levels of FRs in vehicles emphasize their substantial use in vehicle components, and the lack of a transition away from deca-BDE indicates a lag time between regulation and implementation. Further, the ongoing presence of lower levels of lower brominated PBDEs (10-100 ng/g range) may indicate contributions from recycled car parts or substantial debromination of BDE-209.

### **3.21.P-We215 Effect-based strategy for identification and assessment of potential health effects of hazardous organic chemicals in indoor environments**

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People spend most of their time in different indoor environments, such as their homes or transportation vehicles. There is a

concerning lack of information about what hazardous chemicals that could be found in these environments, as well as the effects that they have on the human health. Since additives in plastic can migrate from the material, it is likely that plastic could be an important source of several airborne chemicals found in indoor environments. Polycyclic aromatic compounds (PACs) is a group of chemicals associated with traffic emissions, plastic additives and being formed during manufacturing or recycling of plastics. In this project, chemical characterisation of synthetic materials and indoor air in car cabins will be performed. The aim is to develop methods for screening and identification of hazardous chemicals and their sources. Different groups of PACs, e.g., polycyclic aromatic hydrocarbons (PAHs), alkyl-PAHs and oxy-PAHs will be targeted, as well as other groups such as pesticides and plasticisers. This will be complemented with bioanalytical *in vitro* tests for measurement of different effects, like hormone like effects and genotoxicity. Samples will be collected inside and outside the car whilst driving on the countryside vs in the city, and whilst heating up the car to 70 degrees Celsius in a climate chamber. In addition, materials from the inside of the cars will be extracted, and emission tests of individual materials will also be done to identify sources of chemicals. These samples will be analysed using 1) targeted- and non-targeted chemical analysis, 2) a battery of *in vitro* reporter gene assays covering important mechanisms of toxicity and, 3) effect-directed analysis (EDA) to identify potential toxic airborne chemicals. The results from this study will increase the knowledge about the identity, sources and the combination effects of chemicals present in the car cabin environment as well as appropriate methods to screen for the chemicals.

### **3.21.P-We216 A novel biomonitoring platform with caged lumpfish (*Cyclopterus lumpus*) eggs for spreading of rubber- and plastic-associated chemicals from urban run-off into a harbour area in Central Norway**

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Some rubber and plastic materials are known to contain a high proportion and diverse range of potentially hazardous chemicals, many of which have been shown to leach out and partition to environmental matrices (water, sediments, soils) in laboratory studies. However, knowledge on the fate and bioavailability of these chemicals in the marine environment remains scarce, limiting the potential for conducting accurate and robust risk assessment and effective policy development and implementation. In the present study, we deployed fertilized lumpfish (*Cyclopterus lumpus*) eggs (1 dpf) at two urban harbour areas and a reference site close to the city of Trondheim for 17 days to study uptake of rubber- and plastic-associated chemicals, as well as a wide range of legacy pollutants, using gas chromatography tandem mass spectrometry analysis. We found accumulation of UV stabilizers (benzophenone and benzothiazoles), plasticizers (n-butylbenzenesulfonamide), reagents, and polymer synthesis precursors (bisphenol A, acetophenone, phthalide, and phthalimide) in eggs deployed in the harbour areas. Several of these compounds were detected in concentrations above quantified legacy contaminants in the same study areas. Although high survival was observed in eggs exposed at all sites, a significantly higher presence of embryotoxicity was observed in the harbour-exposed eggs than in the reference site. Further studies are needed to investigate which pollutant(s) are driving the observed embryotoxicity. The current study demonstrates the use of a novel biomonitoring platform using caged fertilized lumpfish eggs to assess the uptake and toxicity of rubber- and plastic-associated chemical pollutants directly in the marine environment.

### **3.21.P-We217 Identifying and Mitigating Hot Spots of Salmon Exposure to Toxic Road Runoff in Metro Vancouver**

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Recent research has revealed that *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone), a transformation product of the rubber antiozonant 6PPD, which is present at high concentrations in car tires, is acutely toxic to some salmonids. In particular, 6PPD-quinone has been linked to coho salmon mortality in streams heavily affected by road runoff.

Our project, Salmonid Toxic Runoff Exposure and Mitigation (STREAM), is investigating the transport and fate of 6PPD-quinone and other toxic road runoff compounds (TRRCs) to water courses used by salmonids, their impacts, and potential mitigation measures to reduce levels in streams. Our objectives are to: 1) identify hot spots of increased salmon exposure to TRRCs, 2) assess the removal of TRRCs from stormwater by green infrastructure and 3) Improve the design of green infrastructure for TRRC removal.

This poster presents preliminary data from the first year of our project for each of these objectives. We will present data from a field survey of salmonid-bearing streams across the Metropolitan Vancouver, a large urban area with populations of sensitive species that our data indicate may be exposed to road runoff. We will also show results assessing the efficacy of stormwater retention ponds in reducing loadings of TRRCs to receiving water bodies, revealing preliminary information on whether they act as secondary sources of 6PPD-quinone to streams. Finally, we will suggest design, operation, and management interventions to improve the ability of green infrastructure systems to reduce loadings of TRRCs to salmon-bearing streams while maintaining their ability to meet water-quantity objectives. Together, the results of our project will help storm water managers, urban planners, and engaged community members protect salmonids across the Metro Vancouver area and beyond.

### 3.21.P-We218 Investigating Alternative Environmental Sources of Toxic Tire Additives

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2-((4-Methylpentan-2-yl) amino)-5-(phenylamino) cyclohexa-2,5-diene-1,4-dione (6PPD-Q) has been shown in recent years to cause mortality in North American freshwater fish species. 6PPD-Q is a transformation product formed from an additive found in rubber tires. The major focus of scientific efforts concerning 6PPD-Q contamination pathways has since been on paved roads. However, over half the road network in Canada consists of unpaved roads. Additionally, employment of recycled tire material is increasing as recycling initiatives mature and expand in scope. The use of recycled material could potentially increase the inputs of 6PPD-Q into the environment beyond paved roads. We hypothesize that (1) unpaved road networks can act as a source for 6PPD-Q in the environment and that (2) recycled tire products have the potential to leach 6PPD-Q under environmental conditions. To investigate this, we collected gravel from the surface of unpaved roads in and around Saskatoon, Saskatchewan, Canada. Chemical extractions and 72-hour leachate studies will be performed on the collected road surface to detect and quantify tire additive concentrations in the leachate. We also performed a long-term leaching study to detect 6PPD-Q that has leached out of the recycled materials under environmental conditions. To accomplish this, we are collecting rainwater and meltwater that has infiltrated through layers of recycled rubber material, then quantifying the 6PPD-Q concentrations found in the leachate. During these studies, we expect to quantify environmentally relevant concentrations of 6PPD-Q in the collected leachate and gravel road dust. Preliminary results suggest that leachate from recycled material contains concentrations of 6PPD-Q lethal to certain fish species. Identifying 6PPD-Q in unpaved roads could lead to unpaved roads being considered as reservoirs for tire particles and related toxic leachates. Furthermore, detecting 6PPD-Q in recycled material leachate could change how recycled materials are utilized in the environment. The results of these novel studies will fill a critical knowledge gap concerning the potential environmental sources of 6PPD-Q and other tire additives.

### 3.21.P-We219 The Fate of 6PPD-quinone in Soil and Water-Sediment Systems using a <sup>14</sup>C Radiotracer

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6PPD-quinone (6PPD-Q), a transformation product of the antiozonant and antioxidant 6PPD (N-(1,3-Dimethylbutyl)-N'-phenyl-phenylenediamine), is believed to be carried from tire and road wear particles (TRWP) into watercourses. Some publications have identified 6PPD-Q as a contributor to Urban Runoff Mortality Syndrome (URMS) of a single specific fish species, Coho salmon.

The potential mobility of 6PPD-Q in soils and sediments has been investigated using the OECD 106 test guideline (Adsorption - Desorption Using a Batch Equilibrium Method) and <sup>14</sup>C-radiolabelled 6PPD-Q. This study is used to indicate how likely a compound is to move through soils.

Additionally, the transformation of 6PPD-Q has been investigated according to OECD test guidelines 307 (Aerobic and Anaerobic Transformation in Soil), OECD 308 (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems) and OECD 309 (Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test). These studies were selected to allow us to gain an understanding of the transformation and partitioning of 6PPD-Q in the key environmental compartments.

Initial results were presented at SETAC North America (Louisville, November 2023). This presentation summarises the findings, including identification and quantification of transformation products generated during these aforementioned studies.

### 3.21.P-We221 Chronic effects of three tire rubber-derived contaminants on zebrafish embryos

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Tires are considered an important source of *non*-conventional plastic particles originated at the tread/asphalt interface. Consequently, about one billion of abraded tires (17 million tonnes) reach the end-of-life every year worldwide. This type of environmental pollution is amplified by the wide plethora of chemicals, adsorbed during the activities of transport means or used as additives in the rubber mixture, which, in turn, could have negative implications on biota. Therefore, the aim of this study was the evaluation of the chronic toxicity (sub-lethal effects) by three tire rubber-derived contaminants in the embryos of the freshwater model *Danio rerio* (zebrafish). In detail, we exposed the selected specimens from 0 to 120 hours post fertilization (hpf) at 1,3-dimethylbutyl-N'-phenyl-1,4-benzenediamine (6PPD), an additive used as antioxidant in tires, 6PPD-quinone (6PPDq), the main 6PPD metabolite, and 1,3-diphenylguanidine (DPG), an accelerator of rubber vulcanization. These three chemicals were tested at the environmentally relevant concentration of 500 ng/L. We certified both the exposure concentrations, as well as the stock solutions (1 mg/L), of selected substances through Liquid Chromatography-High Resolution Mass Spectrometry. During the exposure the eventual acute effects (coagulation of eggs, lack of somite formation and heart breath), as well as sub-lethal abnormalities (scoliosis, development delay, edemas and malformations), were daily registered. Since in all experimental groups no acute toxicity was observed, with an average value of embryos viability of 94.2 ± 1.4 %, we processed the samples for the chronic effect evaluation. We used an integrated ecotoxicological approach of a biomarker suite coupled with gel free proteomics to identify the possible mechanism of action (MoA) of selected chemicals.



### 3.21.P-We222 Tire Related Additive Chemicals in Road Run-Off & Recipient Waters, Snow and Sediment Samples from Six Nordic Countries

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Tire related additive chemicals, such as the antioxidant 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine), one of its transformation products 6PPD-Q (6PPD-Quinone), and the crosslinking agent HMMM (Hexa(methoxymethyl)melamin) can leach out of tires and enter the environment. While there is a growing amount of literature available concerning the occurrence of these chemicals in specific geographic areas and/or specific time intervals (e.g., during rain events), data from large screening studies is currently missing. Here, we present data on the occurrence of tire related additive chemicals in road run-off and recipient waters, snow and sediment samples from Norway, Sweden, Iceland, Faroe Islands, Finland and Denmark. Samples were collected during spring 2023 and were analyzed using liquid chromatography–mass spectrometry (LC/MS).

HMMM was the predominant compound in water samples, including snow samples, due to its relatively high water solubility. On the other hand, 6PPD, which tends to adsorb to particulate matter, was predominant in sediment samples. Tire related additive chemicals were present in all samples, but strong differences in concentrations were observed according to sample types and sampling location. The highest concentration observed for a single compound was 11.5 µg/L HMMM in a road run-off sample. Nordic countries are potentially particularly prone to contamination from tire related chemicals, due to the frequent use of studded tires, which can enhance the production of tire and road wear particles (TRWP). Poor road conditions and the application of road salts in cold climate regions can further increase the emissions of TRWP into the environment and thereby also the emissions of tire related additive chemicals.

### 3.21.P-We223 Application of a Multispecies Approach to Assess the Effects of Car Tires Leachate Toxicity on Freshwater and Terrestrial Environments

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The contact between tyre and road surface causes shear and heat in the tyre. Both processes result in the generation of wear particles. Several studies have suggested that wear and tear from car tyres is an important source of microplastics in the environment. On the other hand, tire materials are complex mixtures, as various chemicals are used during the production of tires. Besides the rubber itself, fillers, reinforcement agents, processing aids, accelerators and retarders, adhesives, and activators may be added. Once released in the environment these particles may undergo leaching processes releasing a cocktail of different chemicals which may cause deleterious effects to the aquatic life. Even if such type of pollution has been widely investigated for marine environments, limited is the knowledge on freshwaters and terrestrial organisms. In this study, tyre material has been ground, size fractionated and leached (1 g/l) under natural like conditions using freshwater and terrestrial compatible leaching medium. A dilution series (0.1 to 100%) was performed, and leachates exposed to the green microalgae *R. subcapitata*, the rotifer *B. plicatilis*, the freshwater protozoan *Euplotes* spp, the zebrafish *D. rerio*, the crustacean *D. magna* and three terrestrial plants (*S. saccharatum*, *L. sativum* and *S. alba*). A battery of different endpoints across the different species including survival and hatching success are assessed, as well as other relevant biomarkers such as oxidative stress, DNA damages and general cell functioning. The LC50, EC10, EC20 and EC50 is calculated for all investigated biological models. Distinctive species-specific pattern of responses is observed at sub-lethal level. This work is expected to help establishing baseline knowledge for supporting the environmental risk assessment of tyre wear particles leachates in the freshwater and terrestrial ecosystems.

### 3.21.P-We224 Echoes of Ecosystems: Comparing Phthalate Exposure in Sentinel Bottlenose Dolphins (*Tursiops truncatus*) Residing in Sarasota Bay, Florida and Barataria Bay, Louisiana

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Phthalates are a class of endocrine disrupting chemical additives commonly used to increase desirable properties of consumer goods (e.g., plastics, personal care products, cleaning products, pesticides). Phthalates are not chemically bound to the products they modify, so they easily contaminate the environment, and have been documented in air, soil, water, and biota. Environmental phthalate contamination has been linked with urbanization and land use. The objective of this study was to compare phthalate exposure between geographically distinct populations of bottlenose dolphins (*Tursiops truncatus*) inhabiting estuaries where phthalate contamination likely differs (potentially related to bay features, such as land use, hydrology, proximity to urban centers, wastewater treatment practices). Dolphin urine collected during catch-and-release health assessments conducted in Sarasota Bay, Florida, USA (2010-2019; n= 51) and Barataria Bay, Louisiana, USA (2011-2023; n= 47) was screened for seven phthalate metabolites: monoethyl phthalate (MEP), mono(2-ethylhexyl) phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), monobenzyl phthalate (MBzP), monoisobutyl phthalate (MiBP), and monobutyl phthalate (MBP). All metabolites were detected in at least one dolphin from both locations except MiBP, which was not detected in Sarasota Bay dolphins. Overall, the percentage of individuals with

detectable urinary phthalate metabolite concentrations was higher in Sarasota Bay than Barataria Bay (~75% vs ~47%, respectively). The most commonly detected metabolites in Sarasota Bay dolphins were MEHP and MEP, while MEHP and MBzP were most common among Barataria Bay dolphins. Among dolphins sampled at both locations, differences in metabolite detection were not observed relative to sex or age class, suggesting widespread phthalate exposure vulnerability. Differences in exposure prevalence and detected metabolite profiles (i.e., MEP vs MBzP) may suggest geographic differences in environmental contamination, potentially attributed to human influence. MEP detection in humans, for example, is often associated with dermal exposure from personal care product use, so environmental exposure may be linked to anthropogenic activity. Additional studies are warranted to investigate potential relationships between exposure variation and habitat utilization to better understand the influence of urbanization on environmental contamination.

### **3.21.P-We225 Phthalates Contamination of Wild Pollinators in an Urban and Peri-urban Context**

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The rising science of urban ecology showed that cities could be wild bee-friendly, pointing out that urban areas can show higher abundance and diversity compared to adjacent rural areas. However, growing urbanization comes with harsher constraints (habitat fragmentation, heat island effect, pollutants...). In the actual context of general pollinator decline, it seems useful to better understand the relative contribution of the different urban parameters to the sustainability of wild bee populations, in order to support this welcome urban biodiversity. Among urban parameters, exposure to urban atmospheric pollutants and their effects on insect health have been poorly investigated. Moreover, recent regulations on transport emissions have changed the toxic profile of the atmosphere of Western cities towards a now majority release of volatile chemicals, among which phthalates are well represented. Proven endocrine disruptors for many vertebrate models, very little is known about terrestrial insects exposed to phthalates except that their cuticle is an efficient trap for these lipophilic contaminants, suggesting that they could accumulate these contaminants when evolving in a contaminated environment. In the present work, we addressed this hypothesis by measuring the cuticular contamination of bumblebees caught in different urban and peri-urban sites. Due to their foraging activity, bumblebee workers make numerous trips back and forth from their nest, within a radius of a few hundred meters, and are therefore considered sentinels of their immediate environment. We measured the magnitude of contamination of bumblebee workers and compared the phthalates mixture they bear on their cuticle with that absorbed by passive sensors installed nearby. Three collection sessions were carried out over the pollination period to study the effect of average temperature. Results confirm the massive contamination of bumblebee populations with ~80% of the total number contaminated with at least one phthalate and ~30% contaminated with more than 5 phthalates, DEHP and DBP being the dominant contaminants. They also highlight the great variability of this type of contamination, not easy to relate to local conditions.

### **3.21.P-We226 Spatial Distributions and Ecological Risk Assessment of Phthalate Esters in the Surface Water of the St. Lawrence River and Estuary in Canada**

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Phthalate esters (PAEs) are a group of chemicals widely utilized as plasticizers in plastic production to enhance flexibility and durability. Additionally, they are used in a broad range of consumer products, including cosmetics, medical devices, and toys. However, as they are not chemically bound to plastics, they can easily escape into the environment and pose risks to ecosystems. Due to the vast global production of plastics, numerous studies have found PAEs to be ubiquitous in various environmental matrices. PAEs are also considered endocrine disruptors and can interfere with the normal functioning of hormones, leading to adverse health effects in organisms. As a result, the United States Environmental Protection Agency (EPA) has designated six PAEs as priority pollutants due to their harmful impacts on human health and the environment. To this date, there is no data on PAEs reported in the St. Lawrence Estuary, the main habitat for the endangered St. Lawrence beluga whale. We also do not know if these chemicals could drain into this system via rivers like the St. Lawrence River. To this end, this study aims to investigate the spatial distributions of nine PAEs in the surface waters of the St. Lawrence River and Estuary and assess their ecological risks in water. Sampling was conducted in summer 2022. Surface water samples were collected from 30 coastal sites and 15 offshore sites in the St. Lawrence River and Estuary. Air and water field blanks were also collected. Samples were analyzed as soon as possible after collection. The aqueous dissolved phase was extracted via solid-phase extraction while the particles were ultrasonically extracted. Analysis was done with GC-MS/MS. Results from the aqueous dissolved phase showed the detection of the nine PAEs and the total PAEs ranged from 6 to 565 ng/L. The concentrations of the coastal sites were higher than those of the offshore sites. Di-n-octyl phthalate, one of the six priority PAEs identified by EPA, was detected in 87% of the sampling sites. An ecological risk study was conducted in water and the results showed that some PAEs (eg., Diisobutyl phthalate (DiBP)), could have a high risk for species like crustaceans and fish. This study establishes a baseline for monitoring PAEs in the St. Lawrence River and Estuary.

### **3.21.P-We227 Associations Between Building Characteristics and Plasticizers in Indoor Settled Dusts**

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Phthalates are plastic additives typically detected among the highest levels in indoor settled dusts. These dusts represent an important potential exposure route to people, especially young children, but can also offer insight into the properties of the indoor environment associated with elevated levels of indoor plasticizers. In this study we profiled house dust in 120 Czech homes for a broad set of plastic additives, including 17 phthalate esters. Eight phthalates were broadly detected, with bis(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate notably detected in all dust samples. Phthalates in dust were strongly dominated by diethyl phthalate, with a median of 1800 ng/g in dusts, and individual concentrations up to 41 µg/g. DEHP was detected in all dusts with a median of 98 ng/g.

To assess the drivers of indoor plasticizers levels, we investigated the association between individual and total phthalates in indoor dusts, and indoor and outdoor home characteristics, including degree of urbanization and population density, type of home or apartment, primary building and flooring materials, and composition of the household. While phthalate levels indoors have been associated, in particular, with the use of PVC flooring, we found that building age and ventilation were most relevant to phthalate levels indoors. In addition, we explored seasonal variations in dust within the homes, in a subset of homes using monthly dust samples, which identified the combined effects of temperature, heating use and seasonal changes in ventilation on indoor plasticizer levels.

### 3.21.P-We228 Dietary exposure of Japanese people to phthalates and their substitutes

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#### Introduction

Phthalates have been widely used as plasticizers for plastic products. In recent years, many countries have imposed restrictions on the use of some kinds of phthalates, especially in the food industry. For a long time, di(2-ethylhexyl) phthalate (DEHP) was the most widely used plasticizer. However, more recently, di(2-ethylhexyl) adipate (DEHA), di(2-ethylhexyl) terephthalate (DEHT), trioctyl trimellitate (TOTM), acetyl tributyl citrate, (ATBC) and 1,2-cyclohexane dicarboxylic acid diisononyl (DINCH) have been developed and used as alternatives to DEHP. Nevertheless, there is little clarity regarding the exposure of Japanese people to phthalates, including these substitutes. In this study, as part of our research on the pharmacokinetics of chemicals derived from food, we measured the dietary concentrations of phthalate-related substances and their substitutes and recorded the results.

#### Methods

We conducted an intervention study in which a totally of 100 healthy men and women were asked to consume the same diet for five days on five separate occasions. The meals were analyzed as yin meals, and after each meal, some water was added to the samples, which were then mixed and lyophilized. The samples were analyzed for DINCH, DEHA, DINA, DEHT, TOTM, ATBC, 2,2,4-trimethyl-1,3-pentanedioic diisobutyrate, and phthalate degradation products (2-ethyl-2-hexanol and texanol) using GC-MS (Agilent 5973-6890) and GC-TOFMS (Agilent 7200 Q-TOF). For substances without corresponding d-body, the samples were analyzed with 2 µg of analyte protectant, PEG300, per injection (1 µL).

#### Results and Discussion

When analyzed without PEG300, the recoveries for substances with its d-body were 85%–105%; however, for other substances, the recoveries were much higher than 100%, with some substances exhibiting recoveries of approximately 150%. When analyzed with PEG300 as an analyte protectant, the recoveries were improved to approximately 100%. The GC-MS analysis showed that DEHP (N = 72) was detected in 99% of the diet samples, with a median concentration of 0.048 µg/g-food per actual weight of the diet samples. Besides DEHP, di-n-butyl phthalate was detected in more than 50% of the dietary samples (detection rate: 67%, median: 0.0061 µg/g-food).

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### 3.21.P-We229 Investigation of the effects of rotenone, bisphenol A and dibutyl phthalate in *Schizosaccharomyces pombe* strains

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Different compounds widely used in everyday products present multiple risks to environmental and human health. Among them, the pesticide rotenone and the plasticizers bisphenol A and dibutylphthalate are very relevant. The aim of this work was to investigate their effects independent of endocrine disruption and complex I inhibition. For this purpose, the fission yeast *Schizosaccharomyces pombe* was selected, an experimental model lacking complex I of the mitochondrial electron transport chain, and estrogenic receptors.

The cytotoxicity, oxidative stress and the possible potentiation of the effects were evaluated with the wild strain. The main mechanisms of action were investigated in strains with deleted genes, to detect stress (*pmk1*, *sty1*) and interference with microtubules (*mph1*) or DNA (*rad3*). The protection by exclusion pumps was evaluated globally and for the specific transporters *Bfr1*, *Pmd1*, *Mfs1* and *Caf5* and also for the transcription factor *Pap1*.

The main results observed were: a) rotenone presented low toxicity in *S. pombe* due to the lack of its main target and because a marked protection by the transporters *Bfr1*, *Pmd1*, *Caf5* and *Mfs1*, being oxidative stress the predominant mechanism of action; b) bisphenol A also induced general stress according to *Pmk1* and *Sty1A*, reducing cell growth and increasing oxidative stress. c) dibutylphthalate presented low toxicity and marked protection by the *Bfr1*, *Caf5* and *Pmd1* transporters. Finally, potentiation of their effects was observed, particularly for rotenone by the fungicide carbendazim and the antimetabolite drug hydroxyurea. Given that the compounds evaluated present complex adverse effects and that their combination may cause potentiation, for their uses, more exhaustive controls and regulations should be considered.

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### **3.21.P-We230 A Comprehensive Analysis of Phthalates Microplastics Effects on Developing Zebrafish: Genomic, Organ Development, and BMP Pathway Insights**

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The escalating global plastic pollution crisis, driven by projections of a two-fold increase in plastic production by 2050, has triggered widespread accumulation of plastic waste, particularly microplastics, in diverse ecosystems. Of particular concern is the leaching of chemicals from these microplastics, with a significant focus on phthalates, commonly used as plasticizers in various products. Phthalates pose a substantial threat to aquatic life and ecosystem health. This study utilizes zebrafish, a well-established model organism for toxicity assessments, to comprehensively investigate the impact of phthalates on aquatic organisms. Zebrafish larvae exposed to three prominent phthalates, DEP (diethyl phthalate), DBP (dibutyl phthalate), and DEHP (di-ethylhexyl phthalate), exhibit reduced survival rates, with DBP demonstrating the highest toxicity among them. These exposures also result in various malformations, including pericardial edema, spinal deformities, yolk sac edema, and tail malformations, with DBP showing the most pronounced effects. The study further reveals that phthalate exposure triggers a significant increase in intracellular reactive oxygen species (ROS) production in zebrafish larvae, with the highest ROS induction in DBP-exposed larvae, correlating with higher mortality rates and malformations. The research extends to examine histopathological consequences in adult zebrafish exposed to DBP and DEP, revealing structural modifications in vital organs, including liver lesions, alterations, necrosis, and cellular degeneration. Additionally, the study investigates genetic expression patterns in zebrafish larvae exposed to phthalates. Several genes associated with organ development, dorsoventral patterning, and cardiovascular development display downregulation in response to phthalate exposure, reinforcing the developmental impact of these chemicals. Beyond these fundamental observations, the study delves into the intricate mechanisms underlying the effects of phthalates. It explores the influence of phthalates on the Bone Morphogenetic Protein (BMP) pathway during zebrafish development. Staining methods, including alizarin, calcein, and alcian blue, are employed to evaluate bone development. These analyses provide valuable insights into how phthalates disrupt bone development and interfere with the BMP pathway in zebrafish.

### **3.21.P-We231 Plastic additives: trophic level ecotoxicity for enhanced Life Cycle Assessment Impact**

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Plastic waste poses a significant environmental challenge, drawing considerable research, media, and policy attention primarily focused on its visible impact. The plastic industry incorporates various additives, such as plasticizers, to achieve desired product properties. Consequently, plastics serve as potential vectors for the release of these additives into the aquatic environment, posing ecotoxicological risks. Despite the prevalence of these additives in aquatic ecosystems, there are substantial knowledge gaps concerning their ecotoxicological effects, especially in the context of Life Cycle Impact Assessment (LCIA). There is a lack of characterization models for additives to assess their environmental impact on the plastic life cycle. In our literature review, we identified ecotoxicity data for 75 additives used in the plastic industry, a very small fraction compared to the extensive range of additives reported. This study aims to address this gap by providing aquatic ecotoxicity data aiming to calculate EF specifically for plastic packaging additives. We conducted a battery of ecotoxicological tests involving species from three trophic levels, to estimate effects such as growth inhibition of algae *Pseudokirchneriella subcapitata*, acute immobilization of invertebrate *Daphnia magna*, as well as the toxicity assessment of the luminescent bacteria *Vibrio fischeri*, following OECD guidelines and the requirements to produce relevant data for LCIA advances. These data are crucial for a better understanding of the environmental impact of plastic additives on aquatic ecosystems, contributing to LCIA by refining and expanding the available sets of effect factors. Advancements in this area enhance plastic life cycle assessment, enabling the identification of toxicity associated with additives and the estimation of their chemical footprint. The findings of this study not only expand the number of covered additives used in plastic packaging but also support initiatives aiming to minimize environmental hazards associated with plastic waste, informing sustainable practices in plastic production.

### 3.21.P-We232 Differences in Modulating Phenanthrene Bioavailability and Toxicity in *Parhyale hawaiiensis* by Microplastics: Short- and Long-Term Effects

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Polycyclic aromatic hydrocarbons (PAHs) and microplastics (MPs) are environmental pollutants that interact with marine organisms and pose unknown risks. MPs sorb PAHs and influence their environmental fate; however, the short- and long-term effects of sorption on PAH bioavailability and toxicity require further investigation. We examined the bioavailability and toxicity of phenanthrene (Phe), a PAH, to determine the effect of sorption on three environmentally prevalent MPs – polyamide (PA), polyethylene (PE), and polyethylene terephthalate (PET) – in the tropical marine amphipod *Parhyale hawaiiensis*. Adult *P. hawaiiensis* (8 – 12 months) were exposed to different concentrations of Phe with and without MPs for short-term (96 h) and long-term (21 d) durations. In short-term exposures, the presence of MPs reduced Phe toxicity, reflected in higher median lethal concentrations (LC<sub>50</sub>), without significant differences among the MP polymer types. Conversely, MPs did not reduce Phe toxicity in long-term exposures based on *P. hawaiiensis* mortality and moulting frequency. Furthermore, behavioural ecotoxicity tests in long-term exposures indicated that the presence of MPs did not reduce the toxic effects of Phe on growth, foraging behaviour, or condition factor indices. These findings suggest a change in Phe bioavailability based on exposure duration, with MPs influencing short-term exposures but exhibiting a limited impact on Phe toxicity in long-term scenarios. The observed changes in sorption-desorption interactions between MPs and toxicants over time highlight the need to further investigate their environmental implications

### 3.21.P-We233 Mixture Toxicity of a Lipophilic and a Hydrophilic Pesticide with Microplastics and a Perfluoroalkyl Substance

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In natural environments, organisms are never exposed to just a single substance, but always to mixtures of chemicals. Ubiquitous substances like microplastics (MPs) and per- and polyfluoroalkyl substances (PFAS) are expected to be found in any global environment, and therefore are always present in the mixture of compounds that organisms are exposed to. This especially is the case in soils, which act as a sink for many chemicals. Therefore, it is important to assess how these compounds may contribute to the toxicity of other xenobiotics to organisms. MPs are considered to contribute to the translocation of xenobiotics into the organism by adsorbing these compounds followed by desorption once the particles have entered the organism, thereby facilitating exposure to xenobiotics. PFAS may also be hypothesized to influence the toxicity of other compounds, by causing immunotoxicity, or induction of cytochrome P450 enzymes involved in the biotransformation of xenobiotics inside the organism, or by affecting the uptake of (lipophilic) chemicals due to their amphiphilic properties. This study aimed to examine the effects of MPs and PFAS on the toxicity of pesticides to the soil dwelling springtail *Folsomia candida*. A hydrophilic pesticide, imidacloprid (log K<sub>ow</sub> 0.57), and a lipophilic pesticide, chlorpyrifos (log K<sub>ow</sub> 4.7), were used to elucidate the effect of lipophilicity on the potential facilitation of exposure by MPs. Single compound toxicity tests were performed to assess the effects of selected compounds on the reproduction of *F. candida*. EC<sub>50</sub>s of linear low density polyethylene (LLDPE) MPs, perfluorobutane sulfonamide (FBSA), imidacloprid, and chlorpyrifos were 2.9%, 1.2 mg kg<sup>-1</sup> dry soil, 0.3 mg kg<sup>-1</sup> dry soil, and 0.1 mg kg<sup>-1</sup> dry soil, respectively. Three ternary mixture toxicity tests were planned to assess the combined effect of MPs and FBSA with either of the pesticides, as well as of FBSA with both pesticides. Test concentrations were designed to range from 0.25 to 4 TU, and by varying the concentration of each component it was aimed at including all binary and ternary mixtures of the four test compounds. The ternary mixture experiments are currently ongoing. Results of two experiments will be presented at the meeting in May 2023.

### 3.21.P-We234 Ecotoxicological effects of ketoconazole combined with virgin and aged microplastics in *Daphnia similis*

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Currently, many studies seek to understand the action of xenobiotic agents on organisms in aquatic environments. Among the xenobiotic agents studied, microplastics are materials often identified in water bodies, which have adsorptive capacity and toxicological synergistic action when associated with other chemical species. Plastics exposed to degrading environmental conditions have altered physicochemical properties, which is called the aging process, generating structural changes with effects on adsorption phenomena and consequently on toxicological effects. Thus, the present study aimed to understand the relationship of toxicological effects that virgin (MPv) and aged (MPE) polyethylene microplastics, associated or not with ketoconazole (CTZ) fungicide, may represent for aquatic organisms, using the *Daphnia similis* organism as the experimental model. The chemical composition and surface morphology of the microplastics were analyzed using FTIR-ATR and SEM techniques, respectively, while the toxicity of MPv and MPE, isolated or associated with CTZ, was determined using oxidative stress biomarkers in *D. similis* under acute test. The physical and chemical changes caused by the aging of the MP were observed, obtaining evidence of the aging of the material, given the appearance of oxygenated groups and greater wrinkled surface. The microplastics with CTZ were more lethal, reinforced by the higher enzymatic activities of superoxide dismutase (SOD), glutathione S-transferase (GST) and malondialdehyde (MDA). Comparing MPv and MPE associated with CTZ, there

was higher mortality by MPv + CTZ than MPe + CTZ, although the latter caused greater oxidative stress and SOD activation. It was seen that the association between microplastics and the drug was more toxic to the organism than each xenobiotic separately, requiring further evaluations to compare damage between virgin and aged plastics.

Keywords: Fresh water. Imidazole. Redox. Toxicity. Polymers.

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### **3.21.P-We235 Weathered Nanoplastics Cause Zebrafish Developmental Stress and Augment Bisphenol A-driven Estrogenicity**

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Plastic pollution has become a critical issue at the global level during the last decades. Plastics can fragment into smaller pieces, to which all living organisms are continuously exposed, through different routes. Plastic fragments in the environment are classified based on their dimension, the smallest being micro- (diameter < 5 mm) and nano-plastics (NPs) (diameter <100 nm). Most laboratory studies in this field use commercial virgin micro/nanospheres when assessing the impact of exposure to these particles on model organisms. However, fragmented plastics in the environment are not perfectly spherical, and vary in their physicochemical characteristics compared to pristine (non-weathered) plastic particles due to exposure to solar, mechanical, thermal, and biological degradation. In this study, we first compared the response of zebrafish embryos to two physical models of polystyrene (PS) NPs, including commercial virgin nanobeads and laboratory-weathered NPs. The laboratory-weathered NPs are originating from single-use plastic products that, after fragmentation, undergo an accelerated weathering protocol. Through morphological, behavioral, and molecular techniques, we observed that weathered NPs more deleteriously affect embryo development, glucocorticoid-dependent stress response, and locomotor activity than commercial beads. As a second step, we investigated if the weathered NPs may operate as a vector to coexisting pollutants, using Bisphenol A (BPA) as a chemical model substance. After assessing BPA sorption capacity on weathered NPs, we exposed the zebrafish embryos to the particles with and without BPA, and we performed a gene expression analysis, which revealed that zebrafish embryos exposed to BPA-sorbed NPs had increased stress and estrogenic responses. Our findings suggest that exposure to PS NPs can impact the development of living organisms, thereby intensifying their stress response. Additionally, using weathered NPs combined with coexisting contaminants could be an effective tool to evaluate the risks associated with exposure to nanoplastics and related pollutants and to simulate human and wildlife exposure scenarios relevant to the environment.

### **3.21.P-We236 Inflammatory-gene expression and DNA damage effects in zebrafish adults after nanoplastic and benzo[a]pyrene exposure.**

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Nanoplastics (NPs) and benzo[a]pyrene (B[a]P) represent emerging environmental pollutants that have attracted significant attention due to their potential adverse effects on ecosystems. Nanoplastics, characterized by their small size and extensive surface area, pose unique challenges in terms of dispersion, transport, and ecological impact. These NPs, often derived from the breakdown of larger plastic materials, can accumulate in several environmental compartments, raising concerns about their implications for aquatic and terrestrial life. On the other hand, B[a]P, a polycyclic aromatic hydrocarbon (PAH), is a well-known environmental contaminant resulting from incomplete combustion of organic matter, such as fossil fuels and tobacco. With recognized carcinogenic and mutagenic properties, B[a]P has been a subject of regulatory scrutiny and research to understand its distribution, persistence, and potential ecological consequences.

In the present study, the toxicological effects of NPs, B[a]P, and their mixtures in zebrafish adults (*Danio rerio*) were investigated. Three different treatments were considered: a) NPs via dietary intake of *Daphnia magna* previously contaminated with 25 nm diameter polystyrene nanoparticles at a concentration of 500 µg.mL<sup>-1</sup>; b) waterborne exposure to B[a]P at 25 nM; and c) a combination of both contaminants. After 28 days and 3 months of exposure, DNA damages were analyzed in fish blood through the comet assay, and the genes associated with inflammation were assessed in the fish liver through RT-PCR analysis.

Results from this *in vivo* bioassay provide insights into the potential consequences of simultaneous exposure to NPs and B[a]P, addressing concerns related to aquatic ecosystems and human health.

### **3.21.P-We237 Deciphering toxicological effects: the interplay between nanoplastics, industrial residues and aquatic environment**

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The persistent discharge and buildup of plastic particles in ecosystems stem primarily from the widespread industrial application of plastics and the insufficient management of plastic waste. In the environment, where various contaminants coexist, plastics may serve as vectors, facilitating their transport to organisms. Nevertheless, a substantial void in knowledge exists regarding the intricate interplay of nanoplastics (NPLs) and co-contaminants. The aim of this study is to investigate whether the toxicological impact of phenmedipham (PHE, herbicide) and amitriptyline (AMI, antihistaminic) on zebrafish (*Danio rerio*) embryos is modified in the presence of polystyrene NPLs. Fish embryonic test (FET), behavior and biomarkers were explored. Analyzed endpoints: mortality, heartbeat, hatching, malformations appearance, locomotor performance, oxidative stress, neurotransmission and energy budget. The combined exposures of PHE or AMI with NPLs did not affect embryos development, showing that the presence of NPLs did not modulate the PHE or AMI toxicity. NPLs induced hyperactivity, PHE induced hypoactivity, and AMI had no effect. The behavioral assessment showed a simple independent action (hyperactivity) for 0.015 mg/L NPLs + 20 mg/L PHE and an interaction effect (hypoactivity) for the 0.015 (or 1.5) mg/L NPLs + 0.03 mg/L AMI. NPLs increased catalase activity and PHE increased glutathione S-transferases activity. On the combination 0.015 (or 1.5) mg/L NPLs + 20 mg/L PHE (or 3 mg/L AMI), cholinesterase activity was inhibited. Additionally, the mixture of 1.5 mg/L NPLs + 20 mg/L PHE increased both catalase and glutathione S-transferases activities. Increased total glutathione levels were found in 0.015 mg/L NPLs + 0.03 mg/L AMI. The joint presence of NPLs and PHE resulted in a greater impact on various biochemical endpoints compared to individual exposures, indicating an elevated effect of the combined exposures. This phenomenon was not observed in the case of the mixture NPLs + AMI. Divergent interaction effects, including no interaction, synergism, and antagonism, were identified between NPLs and PHE or AMI. This study underscores the significance of considering the influence of NPLs on the bioavailability and toxicity of different types of co-contaminants in the evaluation of the environmental behavior and risks associated with NPLs.

### 3.21.P-We238 Hazard Assessment of Polyhydroxybutyrate Nanoplastics and Caffeine on In Vivo and In Vitro Models of *Xenopus Laevis*

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Pollution is a major impacting driver on wildlife populations, especially amphibians due to their high susceptibility to xenobiotics. Plastic contaminants have been a target of extensive risk assessment research, mainly because of their environmental persistence. Polyhydroxybutyrate (PHB), a bio-based and biodegradable polymer, lacks full assessment for its ecological impact, particularly on its nano-sized particles. In addition to its individual exposure threat, nanoparticles may serve as vectors, facilitating the transfer and release of adsorbed contaminants to organisms. Caffeine, the world's most consumed psychoactive drug, is ubiquitously present and prevalent in aquatic environments. Amphibians are both prey and predators of freshwater ecosystems, playing a key role in population dynamics. Nonetheless there is a lack of knowledge regarding amphibians' sensitivity to emerging to contaminants. Thus, this work aims to: assess the individual and combined effects of caffeine and PHB-NPs to early life stages of *Xenopus laevis*; determine the adequacy of *in vitro* assays as non-animal alternatives for first tiers of amphibian aquatic stages risk assessment. Hence, the toxicity of caffeine and PHB-NPs was assessed individually and in mixture, in 96-h assays with embryos and tadpoles of *X. laevis*, and in 72-h assays with A6 e XTC-2 cell lines of *X. laevis*. The present study provides the first experimental evidence of cytotoxic impact initiated by novel bio-nanoplastics and caffeine exposure, either as a single contaminant or in combination, to amphibian *in vitro* models. Caffeine proved strong effects on survivorship, malformations, heartbeat, and body growth, on both early life stages, while PHB-NPs displayed no toxicity on *X. laevis*. PHB-NPs and caffeine co-exposure displayed no apparent interaction on *in vivo* models, while on *in vitro* assays both examples of antagonism and synergism were observed. Embryos were consistently more sensitive than tadpoles to caffeine, with LC<sub>50</sub> of 196 and 226 mg/L, respectively. Live models were more susceptible to caffeine than both A6 (587 mg/L) and XTC-2 (864 mg/L) cell lines, while for PHB-NPs the opposite was observed. Even though PHB is regarded as a biodegradable and biocompatible polymer, our results indicate that PHB-NPs displayed cytotoxic potential. Thus, it is pivotal to further explore the effects of PHB-NPs on *in vivo* models, in order to better understand its suitability as an environmental-friendly alternative.

### 3.21.P-We239 Nanoplastics and Pharmaceuticals: Unraveling the Hidden Ecotoxicological Web

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Plastic waste pollution poses a significant environmental challenge, yet research regards contamination by nanoplastics (NPLs) remains limited. NPLs, apart from their potential toxicity, can also act as vectors for other harmful contaminants, impacting aquatic and terrestrial ecosystems. However, there is a notable knowledge gap in understanding the interactive effects of NPLs on mixtures with pharmaceuticals. The main of this study is to investigate how the toxicological effects of the pharmaceutical diphenhydramine (DPH) changes in the presence of polystyrene NPLs. Considering both aquatic and terrestrial ecosystems, ecotoxicological model species *Daphnia magna* (aquatic compartment) and *Folsomia candida* (terrestrial compartment) were used. Commercial polystyrene NPLs (44 nm) served as the NPLs model. In *D. magna*, immobilization and biochemical endpoints were assessed after 48 hours. For *F. candida*, reproduction, survival, and biochemical responses were evaluated after 28 days and behavior after 48 hours. In *D. magna*, DPH + NPLs showed a synergistic pattern for oxidative damage (increased lipid peroxidation) at ecologically relevant concentrations. The concentration causing a 50% of effect (EC<sub>50</sub>) for *D. magna*

immobilization was 3.9 mg/L for DPH alone. In the mixture, with increasing NPIs concentration, the DPH EC<sub>50</sub> for immobilization decreased to 0.001 mg/L. In general, DPH + NPIs exhibited more pronounced toxic effects than anticipated based on individual exposures, suggesting synergy between the mixture components. In *F. candida*, DPH effects on survival and reproduction were independent of NPIs presence. However, for avoidance behavior, the effects of mixtures depended on NPIs concentration (0.015 mg/kg: interaction - no avoidance; 600 mg/kg: no interaction - avoidance). Glutathione-S-transferases activity increased after DPH + NPIs exposure (synergism), while the rise in lipid peroxidation seen at 0.015 mg/kg NPIs was absent in mixtures. Effects of the binary mixtures varied with endpoints and concentrations. This study revealed interactive effects between NPIs and DPH, leading to harmful impacts on both *D. magna* and *F. candida*. These findings provide valuable insights for informed decisions by society and policymakers concerning plastics and pharmaceuticals, underscoring the importance of environmental risk assessment when considering contaminant mixtures.

### 3.21.P-We240 Contribution of additive-related effects to LDPE microplastics toxicity for aquatic organisms: a case study with model metal and organic additives

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Microplastic particles (microplastics) often contain an unknown quantity of known as well as unidentified chemicals in addition to the polymer material. Intentionally added chemicals may leach from the polymer matrix under environmental conditions, subsequently entering water bodies. There they may exert toxic effects on aquatic organisms and contribute to the overall chemical load. However, studying such effects is challenging due to the complexity of mixtures that are released, as for most commercial polymers the identity and amount of additives are undisclosed.

Here we present an approach to produce well-defined additive-loaded microplastics applicable to subsequent aquatic ecotoxicity testing. We used ZnO, and commercially available LubioÖ products as model additives, both being relevant in industrial applications as UV-stabilizers and polymer antiaging systems, respectively.

A common amount of a nanoscale ZnO (5 wt%, NanoTek) or LubioÖ (3.5 wt%) was homogeneously distributed in LDPE (DOW 410E), and polymer sheets were casted. These were subjected to cryo-milling, yielding micron-scaled irregular shaped particles. Powders were sieved to obtain comparable particle fractions of > 300 µm, 140 µm-300 µm, and < 140 µm for all samples. In addition to 3 different microplastics (additive-free, ZnO loaded, and LubioÖ loaded), also the single additives were tested. Microplastics were produced from unaged polymers as well as from polymers aged under accelerated UV conditions. They were extensively characterized before ecotoxicity testing regarding the leaching of the known additives. We performed toxicity testing with two aquatic ecotoxicity models; freshwater crustacean *Daphnia magna* and protozoan *Tetrahymena thermophila*.

As expected, the ZnO nanoparticles were acutely toxic (induced immobility) to *D. magna* and slightly cytotoxic to *T. thermophila*. LubioÖ additives did not exert high cytotoxicity in *T. thermophila*, whereas a slight toxicity of the LubioÖ was observed in daphnids. None of the microplastics (additive-free / additive-loaded; unaged / aged) was acutely toxic to both organisms. This is an indication that the concentrations of individual additives as used in our study are too low to pose an acute risk to aquatic organisms. Additionally, our results show that the presented approach for preparing microplastics with defined additive content is promising to distinguish the effects posed by microplastics and additives.

### 3.21.P-We241 Do Microplastics Affect the Toxicity of Metals to Duckweed?

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Microplastics (size from 1 µm to 5 mm) have been frequently detected in different water bodies. Because of their unique surface characteristics, they are considered as good carriers of various contaminants in aquatic environment. However, there are few studies focusing on the combined effects of microplastics and metals on freshwater organisms. Under this context, the aim of this study was to evaluate the effect of an environmentally relevant concentration of microplastics on the toxicity of two metals to freshwater macrophyte.

We investigated the individual and combined effect of 10 mg/L polystyrene microplastics with two different sizes (< 100 µm and >100 µm) and two metals (0.1 mg/L cadmium and 1.5 mg/L zinc) on *Lemna minor*. Plant growth, photosynthetic activity, pigment contents were determined after 7 days of exposure. Results showed that individual microplastics promoted growth of *L. minor*, with the larger tested microplastics increasing the fresh weight by up to 26%. Although Cd alone did not inhibit the growth, combination of microplastics and cadmium decreased the plant growth significantly. We noticed that the combination of the smaller microplastics and Cd induced a weaker inhibition of growth and photosystem II activity than larger microplastics and Cd. No significant changes in pigment contents between the Cd alone and the mixture of Cd and



microplastics were observed compared to the control. The combined effect of Zn and microplastics mainly affected photosystem II activity and increased the non-photochemical quenching dissipation compared to Zn alone.

Therefore, the interaction between microplastics and metals and their combined toxicity to aquatic plants need to be considered when assessing the environmental risk of microplastics and metals to freshwater ecosystem.

### 3.21.P-We242 Toxic effects on zebrafish caused by micro- and nanoplastic: a review

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Plastic pollution in the marine environment has become increasingly prevalent, causing devastating effects on a myriad of inhabitants, both small and large. Annually, approximately 19-23 million tons of plastic find their way into the oceans. The breakdown of these plastics results in the formation of micro- and nanoplastic particles (MPs and NPs). Given the ubiquity of sources and their persistence in the aquatic environment, these particles are globally distributed in various environments including some areas far from emission sources. Therefore, the impact of MPs and NPs on aquatic organisms, along with the potential repercussions for ecosystem functioning, has been a topic of discussion for over a decade. For the investigations of the possible environmental consequences of plastic pollution, studies on plenty of different test organisms were conducted such as zooplankton, mollusks, marine worms, and fish. Among these various test organisms, the zebrafish (*Danio rerio*) represents one of the most common test organisms to investigate environmental pollution and more frequently used to investigate the toxic effects of MPs and NPs. However, the challenge lies in synthesizing the increasing volume of new research in this field. This study aimed to combine results of different studies to find consistencies, irregularities, as well as to summarize and to find gaps in the currently available research data using a semi-structured literature research. Results of the study reveal an alarming amount of negative consequences for zebrafish caused by MPs and NPs, such as heart problems, decreased locomotor function, accumulation in brain, liver, pancreas, gallbladder, intestines, and eyes. Noteworthy contributions of this study include the correlation of size of particles with certain toxicological endpoints as well as the importance of the life stage. Additionally, these various scientific data suggest that the lack of clear guideline for studying MPs and NPs effects in zebrafish impedes the creation of a comprehensive overview, emphasizing the need for a shared methodology to facilitate clearer comparisons.

### 3.21.P-We243 Impact of Micro(nano)plastics on Amphibian Cell Lines

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The low cost, versatility, and durability of plastic materials led to its increased production, resulting in its environmental presence. Once in the environment, abiotic and biotic processes have been shown able to promote their degradation/fragmentation to microplastics (100 nm < MP < 5 mm) and nanoplastics (NP < 100 nm). A decrease in size often increases the bioavailability and reactivity of the particles. This becomes highly relevant to the potential impact that small particles may induce on biota. The increased concern over plastic pollution led to a shift in the plastic industry towards the development of bioplastics. Despite their reported biodegradable nature, when disposed of uncontrollably, bio-based plastics will accumulate in the environment and fragment into MP and NP. Whether bio-based plastics are more eco-friendly than conventional fuel-based is still to be proved. Among the organisms that may be affected by MPs and NPs are amphibians, a group of organisms already under anthropogenic pressure worldwide. However, ethical constraints limit the tests that can be employed to understand the hazards of MP and NP to amphibians. Therefore *in vitro* cellular models based on established cell lines are of great significance for the risk assessment of MP and NP. This study aimed to evaluate the cytotoxic effects of micro(nano)plastics of two fuel-based polymers (polymethylmethacrylate (PMMA), and polystyrene (PS)) and a bio-based polymer (polylactic acid (PLA)) on two amphibian cell lines A6 (adult *Xenopus laevis* kidney epithelial cell line) and XTC-2 (tadpole *Xenopus laevis* carcass fibroblast cell line). The selected cell lines were exposed to NP (from 0 to 400 mg/L) and MP (from 0 to 200 mg/L) obtained through laboratory synthesis (PMMA) or fragmentation of larger particles (to produce environmentally relevant shapes – PS and PLA) and the cell viability was assessed, through 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, up to 72 hours. A fluorescent dye (perylene) was added to PMMA NP to track and localize the NPs after their internalization in the cells. No significant effects on A6 cell viability were observed after exposure to PMMA NP, while cell viability decreased after exposure to PLA MP (200 mg/L). XTC-2 viability decreased at the highest concentration of PMMA NP (400 mg/L) containing perylene and PLA MP (200 mg/L), while PS MP induced no significant effects on cell viability.

### 3.21.P-We244 Effects of Field-collected Microplastic Particles on Zebrafish (*Danio rerio*) Embryos

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Given the global increase in plastic production, the assessment of the environmental impact of plastic waste has become a major issue in ecotoxicology. In particular, smaller fragments known as microplastics (MPs) have been detected in multiple aquatic ecosystems, where – due to their small size and poor biodegradability – MPs can easily be ingested by organisms and passed on within food nets. Ingestion of MPs has been speculated to pose not only physical risks, but to also pose chemical risks through sorption and transfer of anthropogenic environmental pollutants such as pharmaceuticals and pesticides. Thus,

the ingestion of MPs has been associated with adverse effects on mortality, growth, feeding activity, endocrine function and the immune system. As many of the mechanisms underlying toxic effects of MPs are still unclear, further research is needed to assess the risk of MPs to aquatic organisms.

As a non-protected vertebrate model, wildtype zebrafish embryos were used to study the biological effects of organic extracts from MPs collected in European coastal marine waters by partners within the JPI Oceans RESPONSE project (<https://www.response-jpioceans.eu/>). The MP samples mainly consisted of a 100-200  $\mu\text{m}$  size fraction of samples collected in the Tyrrhenian Sea, Adriatic Sea and the Bay of Biscay. Organic contaminants included in the MP samples were extracted by hexane. The endpoints investigated comprised acute toxicity, effects on ethoxyresorufin-*O*-deethylase activity by aryl hydrocarbon receptor-mediated induction of cytochrome P450-dependent monooxygenases (CYP1A), acetylcholinesterase activity and changes in the optokinetic response. Taken together, these experiments provide insight into MP-induced toxic mechanisms and effects in zebrafish embryos, which may help to improve the assessment of the environmental hazard and risk by MPs.

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### **3.21.P-We245 Qualitative and Quantitative Assessment of Microplastics Derived from Ship Paint during Hydroblasting: Estimation of Global Microplastics Emissions from an International Research Vessel study**

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As global concerns over microplastic (MP) pollution continue to grow, the International Maritime Organization (IMO) has highlighted the potential risks of MP derived from ship paint, particularly during hull cleaning processes. Despite the growing awareness, there remains a significant data gap in quantifying the extent of MP emissions specifically derived from these cleaning activities. Our study aims to address this data gap by evaluating MP particles generated during hydroblasting, a common hull cleaning technique.

After collecting samples from hydroblasting on globally operating research vessels, we identified the polymers constituting the paint using Fourier-Transform Infrared Spectroscopy (FTIR), and then measured the weight of particles smaller than 5 mm. Each particle was classified as a polymer such as alkyd, acrylate, epoxide, urethane, or polyester/polyether using FTIR. The weight of the paint particles was measured by multiplying the particle's volume with its density. Additionally, the value for the paint's plastic components was cited to be 54.9%. To develop an MP emission factor, we estimated the amount of paint applied by multiplying the wetted surface area with coating film thickness, and the density of the paint. As a result, from the total paint applied (485.2 kg), 60.5 kg (12.5%) was generated as paint, and 33.2 kg (6.8%) as MPs from hydroblasting. The MP emission factor was calculated as 0.00926 kg/m<sup>2</sup>, which is the value of 33.2 kg divided by the Wetted Surface area of 2161.8 m<sup>2</sup>. To estimate the global emission of MPs from ships, we multiplied these emission factors by the total wetted surface area of global ships (326,126,497 m<sup>2</sup>), considering the hull cleaning frequency which is once or twice every five years, corresponding to 0.2 or 0.4 times per year. As a result, the MP emission was approximately 604~1,208 tons in 2023. Additionally, we estimated the global MP emissions from 2011 to 2023, categorizing by ship type, including bulk carriers, oil tankers, container ships, general cargo, and other types of vessels. The quantity of MP emissions has shown a consistent upward trend over the period.

This data is not only used for risk assessment regarding the global MP emissions from ships, but also contributes to the development of ship cleaning regulations. To obtain more accurate MP emission values, analysis by the frequency of hull cleaning and by year of operation is necessary.

### **3.21.P-We246 Exposures to spherical, fibrous, and fragmented microplastics induced alterations of bioaccumulation, microbial immunity, and epigenetic signature in the mussel *Mytilus galloprovincialis***

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Plastic wastes are globally recognized as the fastest-growing source of pollution worldwide and are therefore an environmental concern of high priority. Microplastic (MP) pollution has become a major global concern due to the widespread use and discharge of plastics into the environment. However, very few studies have assessed the potential variations in the toxicity of MPs according to their shape and size. Therefore, our study sought to identify the biotoxic effects of spherical, fiber-shaped, and fragment-shaped polyethylene terephthalate MPs of different sizes at different concentrations on the Mediterranean mussel *Mytilus galloprovincialis*. The survival rate after exposure to small-sized MPs was lower than that observed for the larger type MPs. Bioaccumulation of MPs was different depending on the exposure periods and MP shapes. Interestingly, the fiber-shaped MPs underwent morphological modifications in the mussel body upon uptake. MP exposure also increased the global DNA methylation levels (i.e., an epigenetic signature), expression of the microbiota immunity-related toll-like receptor gene, and alteration of the gut microbial composition in the mussel. These findings indicated that MPs of different shapes and sizes at different concentrations can alter the bioaccumulation sensitivity of mussels according to the exposure periods, and the balance

of gut immunity and epigenetic process. Furthermore, our results demonstrated that MPs of different shapes, particularly fiber types, can undergo morphological modification in mussel tissues, thus posing a hazardous threat.

### **3.21.P-We247 SUSPECT SCREENING OF EMERGING CONTAMINANTS ADSORBE ONTO MICROPLASTICS IN SEAFOOD: A POTENTIAL ROUTE OF HUMAN EXPOSURE**

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Microplastics are highly persistent and ubiquitous, with the ability to accumulate emerging contaminants (ECs). When marine organisms ingest microplastics, these ECs can be transferred through the food chain, potentially reaching humans through the consumption of fish and shellfish. Given that oceans and seas are the primary food resource for humanity, establishing regulations is imperative to ensure food safety for citizens by monitoring the presence of specific chemicals.

This study introduces a novel suspect screening analytical methodology to assess human exposure to microplastic pollutants by identifying ECs in marine foods. The sample treatment involves the use of SUPRAS, representing a paradigm shift in processing due to its numerous opportunities for improving process yields, selectivity, sustainability, and economics. Supramolecular solvents (SUPRAS) are green nanostructured liquids formed spontaneously in colloidal suspensions of amphiphilic compounds through sequential self-assembly and coacervation. SUPRASs exhibit outstanding properties for analyte extraction and sample cleanup, allowing for the removal of major matrix components while effectively extracting the target compounds.

High-resolution mass spectrometry (HRMS) was employed for detection, annotation, and identification of ECs in marine foods. To explore the potential relationship between microplastic distribution throughout the water column and their intake, various edible marine species from different levels of the water column were analyzed using the developed workflow. Microplastics discovered during the analysis underwent a leaching test, and the resulting leachate was examined using SUPRAS-HRMS. Similarly, muscle tissues were examined using the developed workflow for the identification of ECs.

ECs identified at confidence levels 3-1 (Schymanski's scale) in muscle tissue were cross-checked with those identified in the leachate of microplastics to elucidate potential relationships between microplastics and the presence of ECs in edible marine organisms. This study enhances our understanding of the mechanisms of EC migration and the contribution of microplastics to contamination in marine species, thereby assessing the potential risk of exposure to ECs through diet.

### **3.21.P-We248 The effect of PET microplastics on transcriptomic profile of porcine pituitary – an in vivo study**

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Over the years, the environment has been contaminated with millions of tons of plastic. There are different types of plastics that are produced for a single use, and the most dominant include polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyamide and polyethylene terephthalate (known as PET). Microplastics (MPs) and nanoplastics (NPs) have deleterious influence on mammalian endocrine components, including the pituitary gland – organ having an essential role in many physiological functions such as growth, reproduction, metabolism or stress response. Thus, the aim of this study was to determine the *in vivo* effect of PET microplastics on the global transcriptomic profile of the pituitary gland.

The experiment lasted four weeks and was carried out on 8-week-old, sexually immature gilts (n = 15). Animals were divided into three groups: 1) control – receiving empty gelatin capsule, 2) experimental LD – receiving a low dose of PET MPs (0.1 g/pig/day), and 3) experimental HD – receiving a high dose of PET MPs (1 g/pig/day). High-throughput mRNA sequencing was performed on the NovaSeq 6000 Illumina platform.

The study revealed 145 differentially expressed genes (DEGs) in the pituitary of piglets from the LD group (exposed to a low dose of PET). Among these genes, 32 were downregulated and 113 were upregulated. The DEGs were mostly involved in immune response events – leukocyte mediated immunity (*IL12B*, *RAC2*, *CRP*, *IL13*, *GZMM*), T cell mediated cytotoxicity (*IL12B*, *GZMM*), regulation of mast cell activation (*RAC2*, *IL13*). In addition, the study revealed 176 DEGs in the pituitary of piglets from the HD group (exposed to a high dose of PET). The expression of 47 of these genes was downregulated while 129 was upregulated. The DEGs were involved in processes related to neuroinflammation (e.g., *CXCL8*, *IL-13*, *CSF3*, *LIF*, *AHRR*, *GFAP*, *GZMM*), apoptosis processes (e.g., *ELFN2*, *GDA*, *SFTPA1*, *ABHD16B*, *PITX3*), or associated with pituitary function or hormone regulation (e.g., *RXFP1*, *CBLN1*, *LIF*, *AVPR2*).

This comprehensive analysis highlights the complex molecular responses in the pituitary gland exposed to PET microplastics and indicates possible links to inflammation, apoptosis and hormone regulation. These findings recognize the urgent need for further research to fully understand the impact of microplastic pollution on reproductive health and endocrine function.

### **3.21.P-We249 Effect of microplastics in diet on the transcriptome profile of the ovary in sexually immature gilts**

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As plastics have recently become widely used in industry, agriculture, and daily life, the production of plastics and their products continues to increase dramatically. When plastic waste enters the environment, it breaks down into small pieces (particles < 5 mm) called microplastics (MPs). It was reported that MPs could pass through the intestinal barrier and enter into the circulatory system as well as accumulate in various tissues, including those of the reproductive system. Therefore, the aim of this study was to describe the effects of MPs on the ovary.

The *in vivo* experiment was performed on 8-week-old immature gilts (n = 15) divided into following groups: 1) control, 2) group treated with a low dose of PET MPs (0.1g/animal/day) for 4 weeks, 3) group treated with a high dose of PET MPs (1g/animal/day) for 4 weeks. The transcriptomic profile was analyzed by RNA-Seq.

The study demonstrated that the low dose of PET MPs affected the expression of 12 genes (3 downregulated while 9 upregulated) in the ovary. According to GO analysis, the differentially regulated genes (DEGs) were engaged mostly in process related with apoptosis (e.g. *ADAMTS2*, *PRLR*, *SOX4*). The KEGG analysis showed that the DEGs were involved in the regulation of PI3K–Akt signaling pathway, oxytocin signaling pathway and prolactin signaling pathway. In turn, the high dose of PET MPs altered the expression of 146 genes (103 downregulated while 43 upregulated) in the ovary. The DEGs were engaged for instance in the regulation of inflammatory response (*IL-17B*, *CXCL9*, *CXCL10*) or apoptosis process (e.g. *SOX2*, *ATO1*, *PIP5K1L1*). In addition, the KEGG analysis revealed that the high dose of PET MPS induced changes in the expression of genes regulating steroid hormone biosynthesis, chemokine signaling pathway and

cytokine-cytokine receptor interaction.

The results of this study comprehensively describe for the first time the effects of different doses of PET MPs on immature ovaries. The PET MPs alters genes involved in key regulatory processes in the ovary, such as signal transduction, apoptosis, immune response, and hormone synthesis. Our study provides a basis for further exploring the molecular mechanism of reproductive disorders induced by exposure to PET MPs in females.

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### **3.21.P-We250 PET microplastics affect metabolism of neurotransmitters in the hypothalamus**

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The escalating global production of plastics has led to widespread environmental pollution. Microplastics (MPs), small particles created when plastics break down, are a growing problem that has been detected in numerous consumer products. Our recent study shows that PET plastic particles can be transported by extracellular vesicles (EVs), which are crucial mediators of intercellular communication. In view of the potential infiltration of plastic particles via EVs into the central nervous system, we decided to determine the effect of PET MPs on the hypothalamus – pivotal regulator in the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes.

The experiment was performed on immature gilts (n = 15). Animals were divided into three groups: 1) control, 2) group receiving a low dose of PET MPs orally (0.1 g/pig/day), and 3) group receiving a high dose of MPs (1 g/pig/day) for 4 weeks. The transcriptomic profile of hypothalamus was determined using RNA-Seq method.

The analysis revealed 82 differentially regulated genes (DEGs) in the hypothalamus of piglets that received a low dose of PET MPs. The GO analysis assigned these DEGs to processes such as inositol phosphate biosynthetic process (*CD244*), regulation of ERBB signaling pathway (*PDE6H*) or receptor recycling (*TRATI*). In turn, a high dose of PET MPs changed expression of 128 genes. These DEGs were assigned to processes such as catecholamine metabolic process (*DBH*, *PNMT*), cytokine

production (*CD244*, *C5AR2*, *CLEC7A*). Furthermore, KEEG analysis showed that these DEGs were engaged in pathways such as tyrosine metabolism (*DBH*, *PNMT*), Cushing syndrome (*CYP11A1*, *STAR*, *CYP21A2*) or steroid hormone biosynthesis (*CYP11A1*, *CYP21A2*).

These results suggest that exposure to PET MPs induce changes in gene expression in the hypothalamus, affecting processes related to neurotransmitter synthesis, immune response, and endocrine function. The findings contribute to our understanding of the molecular responses to microplastic exposure.

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### **3.21.P-We251 The chemical composition of oxo-“degradable” plastics and their use in Switzerland**

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Oxo-“degradable” plastics (oxo-plastics) are plastics containing pro-oxidant additives that allegedly promote fragmentation and subsequent biodegradation. However, oxo-plastics, in reality, do not completely biodegrade under environmental or industrial composting conditions, thus contributing to microplastic pollution. Moreover, they can disrupt recycling processes. The Single-Use Plastics Directive in the European Union prohibits the placing on the market of “all plastics containing additives that, through oxidation, lead to fragmentation” (therewith targeting oxo-plastics). In this study, we investigated the occurrence of oxo-plastics in Switzerland and their chemical makeup based on a literature and patent review as well as XRF screening of real samples.

In Switzerland, intentional use of oxo-plastics is not widespread, as larger stakeholders are environmentally conscious and intentionally avoid such products. However, smaller users and distributors of intentional oxo-plastics products exist. Accessing information on oxo-plastics material flows is difficult, as manufacturers and stakeholders do not provide data, and trade statistics do not reveal imports of pro-oxidant additives or oxo-plastic products.

Many substances can act as pro-oxidant additives. The assumption of Fe, Mn, and Co salts being the main pro-oxidant additives is based on limited primary literature. Furthermore, several commercial pro-oxidants also have other functionalities in polymers, thus unintentionally rendering some conventional plastics as “oxo-plastics.” The difficulty in differentiating conventional plastics from oxo-plastics was also observed in the XRF screening, where no differences between oxo-plastics and conventional plastics could be observed.

Based on the study's findings, it is recommended to establish a precise definition for oxo-plastics, considering the multi-functionality of many “pro-oxidant” additives. Additionally, the development of practical methods for identifying oxo-plastics is crucial. Implementing regulations on labeling degradable products may contribute to proper disposal practices.

### **3.21.P-We252 Beyond the Surface: Spotlight on Plastic Metal(loid) Additive Leaching and Unseen Contaminants**

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It is known that the leaching of plastic additives, not chemically bound to the polymer backbone, accelerates due to the sunlight-induced weathering of plastics. As a result, these compounds, which in some cases are harmful to the environment, can be increasingly released e.g. from micro plastic particles - resulting in enhanced hazard potential. Since the mechanisms of how fast and to which extent additives migrate out of polymers are complex, it is important to be able to clearly differentiate between the actual leaching of additives and the release of other (micro) contaminants, such as impurities introduced during production or other metal(loid) inclusions.

For this study, eight plastic consumer products (CPs) covering three polymer types (PE, PET and PVC) were subjected to accelerated weathering and leaching in double-distilled water for ten days under ultraviolet (UV) light and the chemical composition (with emphasis on the metal(loid) fingerprint) of the UV leachates was compared with the multi-elemental fingerprint in the corresponding dark control (DC) samples. In addition to these commercial materials, custom-made plastic samples (CMs) with known and well-defined additive profiles were analyzed after application of the same leaching protocol.

Results showed both CPs and CMs exhibited increased UV leachate concentrations by a factor of 0.1 – 1276.6 compared to DCs, indicating weathering effects from simulated sunlight. Antimony (Sb) concentrations exceeded EU Drinking Water Framework Directive limits, raising concerns. However, CP data alone couldn't confirm if leached Sb solely originated from additives like SbO<sub>3</sub> (flame retardants) or other sources. However, a comparison of CP and CM results revealed additives may not be the sole cause of elevated leachate concentrations, emphasizing the need to assess releases of other contaminants from plastic products beyond additives.

### 3.21.P-We253 Linking Composition to Toxicity in UV-Weathered Plastic Leachates: An Assessment Using Diverse Cell Lines

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The ultraviolet (UV) component of solar weathering significantly influences the leaching behavior of plastics and the photo-transformation of plastic additives. This study explores the correlation between UV-induced compositional changes in plastic leachates and their consequent toxicity. We analyzed leachates from eight consumer plastics exposed to UV radiation and dark conditions using liquid chromatography-mass spectrometry (LC-MS). The leachates were then assessed for toxicity in five human cancer cell lines: liver, skin, lung, colon, and breast tissues. Our analysis identified 12 additives belonging to classes such as flame retardants, plasticizers, antioxidants, and stabilizers. The occurrence of these additives varied distinctly with the type of plastic and treatment condition (UV vs. dark). UV exposure altered the chromatographic profiles of the leachates, by increasing complexity and introducing new peaks at lower retention times, suggesting the formation of more bioavailable transformation products. Further investigation in the US EPA Comptox database revealed that the identified additives exhibit significant activity across various cellular assays, indicating potential biological effects ranging from endocrine disruption to impacts on liver metabolism. Laboratory toxicity assessments of leachate samples showed differential toxicity across cell lines, with the liver cell line (HEP G2) showing the highest susceptibility and the colon cell line (HCT-116) the least. This variance highlights the importance of exposure pathways, where liver cell lines, indicative of systemic toxicity and metabolism, exhibiting greater susceptibility to leachate toxicity compared to colon cell lines, which represent gastrointestinal exposure. Notably, the presence of a high number of detected additives in some plastic products did not always correspond to increased toxicity, suggesting that while parent additives may contribute to toxicity, other factors, such as transformation products, (non)microplastics, inorganic components, and fractions that are not amenable to LC, could play more significant roles in the observed toxic effects. Taken together, this study underscores the critical need for comprehensive analytical approaches to unravel the complex interactions between UV-weathered plastic leachates and biological systems for better environmental and health risk assessments.

### 3.21.P-We254 Are Marine Plastics a Sink or a Source of Organic Chemicals in the North Pacific Ocean?

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Plastics contain a diverse mixture of additives, processing aids, and other organic chemicals, partly of concern, to meet the desired material performance. Once emitted to the aquatic environment, these chemicals can leach from the polymer. In contrast, the high sorption capacity of some polymers, e.g. Polyethylene, renders plastics a passive sampling phase when exposed to environmental matrices. Hence, when floating in the ocean, marine plastic litter constantly (de)sorbs organic compounds from/into the surrounding sea water until equilibrium is reached. In this study, we evaluated if marine plastics in the North Pacific Ocean represent a sink or a source of organic contaminants in ambient waters. For this purpose, marine plastics were sampled in the Pacific Ocean during the expedition SO268/3 of RV SONNE and, if identifiable, matching new products were purchased.

We investigated the co-occurring processes of chemical (de)sorption by effect analysis of chemical mixtures that were solvent-extracted from field-weathered marine plastics and dosed into different cell-based reporter gene bioassays. The observed effects were compared to those from solvent extracts of new plastic products corresponding to the field samples. Moreover, the new plastic products were artificially weathered in a custom-made weathering wheel under UV light and Dark Control conditions. The retrieved plastics were solvent-extracted, and the aqueous leachates were up-concentrated by solid-phase extraction. Both types of extracts were dosed into the bioassays. Finally, chemical analysis of organic pollutants and plastic-associated chemicals was performed by LC-HRMS (Q-Exactive Plus quadrupole-Orbitrap) with subsequent effect modelling to identify mixture toxicity drivers.

The results showed a diverse picture of reporter gene induction caused by either field-weathered plastics (presumably by sorbed chemicals and remaining additives) or by the new products (presumably caused by additives), depending on the product type. Samples for outdoor exposure appeared to be a source of (elevated) effects, whereas, e.g., food-contact material tended to sorb organic chemicals from the surroundings. By chemical analysis and subsequent modelling we could partially explain the observed effects. Our study underlines the relevance of weathering for the leaching of chemicals of concern from marine plastics.

### 3.21.P-We255 Post-consumer recycling plastics (PCR), a safe alternative to pristine plastic bags? A case study: Degradation and toxicity of PCR vs pristine plastic bags.

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The production and use of plastics has increased consistently over the past seven decades, leading to increased levels of plastic pollution across the globe. Accordingly, growing public and political awareness has led to many policy responses, including the ongoing negotiations of the global plastics treaty. Among a range of mitigation actions to reduce the quantity of newly produced plastics released to the environment, recycling of plastic waste has emerged as one of the viable options. From a circularity perspective, recycled materials appear to be a good alternative to the use of fossil-fuel derived pristine plastics to produce plastic consumer products, as well as decreasing the overall volume of plastic waste. However, knowledge remains limited on the degradation rates and mechanisms of the post-consumer recycled plastics (PCR) and their potential impacts on aquatic organisms. In this study, we investigated and compared the impacts of simulated UV (sunlight) exposure on the photodegradation of pristine and PCR (printed and non-printed) polyethylene plastic bags, as well as the potential toxicity of partially photodegraded microplastics on *Daphnia magna*. From an ecological perspective, *D. magna* is a key species in freshwater environments, including Norway. The plastic bags (pristine, PCR, PCR printed) were artificially degraded in a UVC chamber. Microplastics (non-degraded and degraded) were characterized using FTIR and Coulter Counter to investigate the influence of the recycling process on its degradation. The chemical composition (non-intentionally added substances and additives) before and after degradation was assessed by non-target screening of methanol extracts using UPLC-MS/MS and direct analysis of particles by TD/py-GC-MS. Toxicity studies were performed following OECD 211, by exposing *D. magna* to extracts of non-degraded or degraded plastic bags (pristine and PCR). This study is expected to contribute to a better understanding of the impacts of the recycling plastic process on the durability and impacts of recycled plastics in terms of their degradation and toxicity.

### **3.21.P-We256 Aquatic Toxicity Assessment of Chemical Mixtures from Recycled Plastics**

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The recycling of synthetic plastic products is offered as one of the potential solutions to the global plastic crisis although there are several environmental issues concerning the plastic recycling industry. Recycled plastics can contain thousands of chemicals including non-intentionally added substances and contaminants such as traces of pharmaceuticals or pesticides. In this study, we assessed the aquatic toxicity of high-density polyethylene (HDPE) recycled plastic pellets coming from 11 facilities in the global south. A Liquid chromatography High Resolution Mass Spectrometry (LC-HRMS) with target analysis gave an indication of the substances contained in the plastics. The compounds within the plastics were extracted by using hexane, acetonitrile, and methanol. Zebrafish, Chironomid larvae and the phytoplankton *R. subcapitata* were then acutely exposed to two DMSO extract concentrations equivalent to 16g/L and 3.3g/L of plastics. Nine of the 11 plastics showed toxic effects in at least one model organism. Phytoplankton was the most sensitive organism, affected by 7 of the 11 extracts when exposed to the highest concentration and by 4 of the extracts at the lowest. Chironomid larvae were also affected. One of the chemical extracts in particular, caused 88% mortality of the larvae. No apparent deleterious effects were observed in Zebrafish embryos, exposed at the embryo stage.

Our results suggest that the increased use and subsequent dispersion in the environment of recycled plastics has the potential to cause disruptive effects on phytoplankton and invertebrate populations. This study could have management implications regarding the import of recycled plastics.

### **3.21.P-We257 Assessing the Ecotoxicological Impact of Plastic-Associated Chemicals from Consumer Products on the Marine Environment: Using In Vitro Studies with Halibut (*Hippoglossus hippoglossus*) Hepatocytes**

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The increasing amount of microplastics resulting from fragmentation and degradation of marine litter are well documented, but the environmental risks of plastic-associated chemicals are emerging as a much lesser understood threat to the marine environment. The aim of this study was to investigate the toxic effects of plastic-associated chemicals in a marine fish species relevant to the Norwegian environment. To address this effectively, a reproducible, cost-effective and ethically, New Approach Methodologies (NAMs) using *in vitro* methods was implemented using primary liver (hepatic) cells from the Atlantic Halibut (*Hippoglossus hippoglossus*). The toxicity of leachates originating from five different consumer products were assessed, namely shoe soles, balloons, "end-of-life" tyre granulates, washing gloves and a polyethylene terephthalate (PET) bottle. These consumer products were chosen based on previously identified toxicity (shoe soles > balloons > tyre granulates ≈ washing gloves > PET bottle) in marine organisms at different stages of development (embryo, juvenile, adult) and trophic levels (bacteria, algae, copepods, oysters, mussels, sea urchins and fish). The primary hepatocytes were exposed for 48 and 96 hours to the five leachates, after which effect responses as cell viability, endocrine disruption, oxidative stress and detoxification were assessed. Chemical analyses using non-target Gas-chromatography-Mass-spectrometry and Inductively Coupled Plasma Mass Spectrometry were also performed on the leachates, identifying the presence of several plastic-associated chemicals in each product. The outcome of this study will further fill the data gaps on the toxic mode of action of chemical additives in fish liver cells, further supporting the hazard and risk assessment data produced in the MicroLEACH project. The MicroLEACH project was funded by the Norwegian Research Council, project. No. 295174.

### 3.21.P-We258 Comparative Toxicity of Conventional versus Compostable Plastic Consumer Products: an in-vitro Assessment

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The accumulation of plastics in the environment, and in particular of single-use items, is one of the greatest threats caused by human activity. In recent years, biodegradable/compostable plastics (BPs) have been promoted as green alternatives to reduce plastic waste pollution through on-site biodegradation. However, few studies have investigated the toxicity of BPs in ecosystems and the consequences of incomplete degradation in the environment. This study evaluates the toxicity of the methanolic extracts of BPs compared to conventional plastics (both virgin and recycled), and explores the potential influence of plastic photodegradation and composting on toxic responses using a battery of in vitro assays conducted in PLHC-1 cells. The extracts of BPs, but not those of conventional plastics, induced a significant decrease in cell viability (< 70%) in PLHC-1 cells after 24 hours of exposure. Toxicity was enhanced by either photodegradation or composting of BPs. Extracts of conventional plastics, and particularly those of recycled plastics, induced 7-ethoxyresorufin-O-deethylase (EROD) activity and micronucleus formation in exposed cells, indicating the presence of significant amounts of CYP1A inducers and genotoxic compounds in the extracts, which was enhanced by photodegradation. These findings highlight (a) the importance of investigating the effects of degradation mechanisms, such as sunlight and composting, on the toxicity of BPs, and (b) the need to investigate the composition of newly developed formulations for BPs, as they may be as harmful as the conventional ones.

### 3.21.P-We259 Impacts of conventional and alternative plastic leachates on *Hordeum vulgare*: a comparative study in soil and hydroponic environments

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There is a current shift in agriculture towards hydroponics to reduce land and water use and address global food demand. Despite the increased yields offered by hydroponics, concerns have emerged regarding potential adverse effects of polymer leachates, released from plastic agricultural equipment upon degradation, on plant growth, particularly within hydroponic systems. Additives (leachates) from biodegradable polymers have received little consideration. Chemical compositions of biodegradable plastics are often kept confidential by manufacturers, but evidence suggests that during degradation, these plastics can release toxic additives into soil ecosystems. This study focuses on the impact of plastic leachates on spring barley (*Hordeum vulgare*) in soil and hydroponic systems. The leachate of selected plastics (conventional: PE, PP; alternative: biodegradable PE, PLA; and Control) are tested for their effects on the development of spring barley. Leachate concentrations are set at 0.25% (w/v) to mimic lower environmental concentrations. The hypothesis posits that barley seeds exposed to leachate from plastics at germination stage and throughout early plant development stages will exhibit reduced growth. Two experiments are conducted: a 30 day edaphic study, focusing on germinating seeds in leachate from plastics and transferring them to uncontaminated soils, while an 8 day hydroponic study, examined seeds germinated under normal conditions and exposed to plastic leachate in a hydroponic setup. Both experiments assessed effects on plant growth, biomass, chlorophyll content, and oxidative stress. Preliminary findings indicate that, in the edaphic study, leachates of conventional plastics decreased shoot growth by 11%, while leachates of alternative plastics led to a more substantial reduction of 22-23%. In the hydroponic study, leachates resulted in a 29-45% reduction in shoot biomass and an 11-70% reduction in root biomass. Multivariate analyses will be employed to establish relationships between chemical composition of the leachates and observes responses for each plastic type. This research contributes valuable insights into the potential ecological risks associated with plastic leachates in agricultural systems. The outcomes of this study aim to contribute to the ongoing discourse on mitigating the environmental impact of plastic pollution in modern farming techniques.

### 3.21.P-We260 Sex hormone disruption potentials of several biodegradable plastics of human use in H295R cell lines

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Biodegradable plastics are generally recognized as a safer and more sustainable alternative to petroleum-based plastics. While some studies have reported the presence of endocrine-disrupting chemicals in biodegradable plastic products, current knowledge on the endocrine disruption effects and mechanisms of biodegradable plastics remains limited. In the present study, we utilized human adrenocortical carcinoma (H295R) cells to evaluate the sex hormone disruption potential of major biodegradable plastics manufactured for direct human contact. Biodegradable plastics tested in this study, intended for human use, included a sanitary bag (n=1), hygiene gloves (n=2), a plastic cup (n=1), a straw (n=1), and a plate (n=1). These products were made from plastics such as polybutylene adipate terephthalate (PBAT), polylactic acid (PLA), and a PBAT+PLA polymer. Products made from high-density polyethylene (HDPE) were included for comparison. The products were shredded into small pieces and extracted using acetone. Sex hormone levels were measured after exposure to the extracts of these samples. Moreover, expression levels of key genes involved in sex hormone synthesis were measured, including *star*, *hmgr*, *cyp11a1*, *cyp11b2*, *cyp17a1*, *3βhsd2*, *cyp19a1*, *cyp21a2*, *17βhsd1*, and *17βhsd4*.

In H295R cell experiments, exposure to eight plastic extracts generally led to increased 17β-estradiol (E2) levels. However, statistical significance was not always observed. Particularly, the PLA plastic cup extract exhibited a significant 1.46-fold



increase at the highest concentration (5 mg/mL) compared to the solvent control. For testosterone (T) levels, a significant decrease was observed in HDPE sanitary bag and PBAT hygiene gloves extracts. For most products, a significant upregulation of the *cyp19a1* gene was observed in H295R cells.

The results of this study demonstrate that biodegradable plastics may influence sex hormone homeostasis, potentially through alteration of aromatase activity. Effects toward anti-androgenicity observed in the biodegradable plastics were similar to those reported for some phthalates, typical components of PVC plastics. Further investigations are warranted on the environmental health implication of this *in vitro* observation.

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### 3.21.P-We261 Characterising the Chemical Additive Content of Agricultural Plastic Mulch Film

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The cultivation of arable land presents unique regional challenges, such as crop demand, climate pressures or limited economic capacity. The use of plastic mulch film is a globally adopted agricultural practise that can aid in relieving such challenges. Mulch film is a 'plasticulture' application where film is stretched over the soil surface to maintain a humid microclimate at the crops / fruits soil-root zone, whilst protecting seedlings from pests. However, the introduction of mulches to agroecosystems comes with an anthropogenic chemical load, whose ecotoxicological legacy is yet to be assessed. Therefore, extraction and analytical methods were developed to characterise both the polymer and organic additive content in commercially available mulches, intended for field application.

Methods were developed for a low-density polyethylene (LDPE) mulch and a biodegradable polylactic acid (PLA) / polybutylene adipate-co-terephthalate (PBAT) blend. Proprietor confidentiality meant no information was available on the additive content of the films. A non-targeted characterisation approach was therefore adopted, which also allowed for the identification of non-intentionally added substances (NIAS). Microwave assisted solvent extraction and dissolution-precipitation were used to retrieve the additive content from the LDPE and PLA/PBAT mulch respectively. Extracts were analysed using gas chromatography-mass spectrometry (GC-MS) and spectral libraries, to characterise the extractable content of the films. Thermal desorption (TD)-GC/MS was additionally tested for comparable qualitative screening of additives. An array of chemicals were identified including: plasticisers (citrates, adipates), slip agents (fatty amides), antioxidants (hindered phosphates, hindered phenols), antistatics (fatty esters), lubricants (fatty alcohols, alkanes) and transformation products of both additives and polymer. Cyclic oligoesters were especially abundant polymer derivatives in the PLA/PBAT mulch, with comparable total concentrations ( $3.03 \pm 0.03 \text{ mg g}^{-1}$ ) to the extracted additive content ( $4.37 \pm 0.12 \text{ mg g}^{-1}$ ). Additionally, confirmation of the mulches polymeric compositions, or revelation of unreported polymeric components, was achieved through fourier transform infrared (FTIR) spectroscopy and <sup>1</sup>H nuclear magnetic resonance (NMR). Ongoing research investigates the lifetime and transformation of mulch film derived organics in agricultural soils, at environmentally relevant concentrations.

### 3.21.P-We262 Mulches in Agriculture: Tackling Contaminant Transport to Soil and Crops

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Mulching is an agricultural technique aiming at improving crop quality; this practice often involves the use of polyethylene based films, that typically contain additives such as phthalates known to disrupt various endocrine functions.

The potential environmental and health hazards posed by plastic mulching arise from the possible transfer of the pollutants carried by these films to the soil and subsequently to soil biota and edible crops.

In response to these challenges, biodegradable plastic mulches have been produced as an alternative. Their biodegradable nature allows them to remain in the fields, contributing to a reduction in plastic waste and in the costs associated with collection, treatment, and disposal. Despite these advantages, concerns arise regarding the potential release of substances from biodegradable mulches, raising questions about their impact on biodiversity and human health.

To assess the impact of mulches on contaminant transport, we analyzed soil and crop plants exposed to different mulching materials.

A small field experiment was conducted to replicate realistic mulch usage conditions where strawberries plants were cultivated using four different types of mulches, biodegradable and non. Soil and strawberries were collected for GC-MS analysis and are currently undergoing an extraction process that will enable us to detect and quantify phthalates, acetyl tributyl citrate and other plastic additives.

Our results will determine whether biodegradable and non-biodegradable mulches pose risks to agricultural soils and whether there are significant differences between them in terms of contaminant transport.

### **3.21.P-We263 Metallic Additives Leaching and Extractions from Biodegradable Plastics Used in Fishing Gears and Potential Materials: Are They Truly More Environmentally Friendly?**

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Additives are chemical compounds added into polymer synthesis to enhance performance, functionality, and properties of polymers. Each additive plays a different role in improving the final plastic product. Additives are categorized into four groups, namely functional additives, colorants, fillers, and reinforcements. Since they are not chemically bounded to the final material, additives could leach out of it. More focus has been given to organic additives in comparison to metallic ones due to their affiliations to chemical toxicity, with some falling into the same groups as persistent organic pollutants. However, in several studies, it was observed that while organic additives reached an equilibrium after approximately 14 days, leaching of metallic additives showed an increasing continuance. Metallic additives are cheaper than organic ones, with better heat and weathering resistance properties. Although certain metals have been banned from use, due to recycling of plastics, these toxic metals could still be present in trace amounts in newer products. Given their durability, lost fishing gears made of nylon could remain in the ocean for up to 600 years. In addition to visible impacts such as ghost fishing or ingestion, leaching of toxic additives is yet another potential problem that could intensify throughout the food chain. Biodegradable have increasingly been discussed as potential replacement materials for nylon with the advantage of a significant reduction of environmental impacts in case of loss. This study aimed to investigate the leaching pattern of metallic additives, and the total metallic additives content in nylon and five other biodegradable plastics. Of these, two materials, namely polybutylene succinate co-adipate-co-terephthalate (PBSAT) and Poly(butylene succinate-co-adipate) (PBSA) have been produced as fishing gears and have been tested in several fishing trials. The other three polymers, namely polybutylene succinate 1 and 2 (PBS) and polycaprolactone (PCL) are potential materials or potential blending materials for fishing gears given their individual physical properties. Each one of them was microwave digested at an elevated temperature to quantify the total metallic additive presence. Five different leaching experiments ranging from 6 hours to 31 days were set up at two different temperatures for each material at a concentration of 1 gL<sup>-1</sup>. Both leachates and extracts were diluted prior to analysis and quantification using ICPQQ-MS.

### **3.21.P-We264 Biofilms enhance the adsorption capacity of Cd on weathered microplastics generated from mulching material (both biodegradable PLA and conventional PE)**

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Biodegradable polylactic acid (PLA) mulch has been developed to replace conventional polyethylene (PE) mulch in agriculture as a response to growing concerns about plastic pollution. Under some management practices mulches are ploughed into the soil at the end of their use which can lead to the production of plastic fragments including microplastics (MPs). Microplastic mulch residues can also form following degradation of the mulches due to exposure to UV radiation and the weather. Cadmium (Cd) contamination of agricultural soils is an ongoing challenge due to the use of Cd-bearing phosphate fertilisers. Cd can adsorb to MPs. During MP formation, exposure in the natural environment can lead to the formation of biofilms on MPs, which may further impact their adsorption of contaminants. The aim of this study was to investigate the adsorption of Cd on both pristine and naturally weathered MPs (PLA and PE) generated from mulching material. We determined the biofilm content of the MPs to help explain our results. Therefore, large sheets of PE and PLA mulches were prepared and hung outside to age naturally from 3rd April in 2022 to 2nd August in 2023. Both pristine and weathered mulches were cut into small pieces using scissors, shredded to generate MPs using a blender and sieved to less than 2 mm. The PE formed platy particles whilst the PLA formed fibres. This generated the following sample types: pristine PE pieces (PPE), pristine PLA fibre (PPLAF), weathered PE pieces (WPE) and, weathered PLA fibre (WPLAF). Pristine and weathered PLA mulches were also cut to produce similar shaped particles to the PE using scissors (pristine PLA pieces, PPLAP, and weathered PLA pieces, WPLAP). 50 images of each MP type were captured using microscope and then analysed using imageJ. We carried out adsorption experiments to investigate the potential for different MPs to adsorb Cd. In the subsequent experiment, for each MP type, triplicate samples of four pieces of MP were analysed to determine the amount of biofilm on their surface by the crystal violet staining method. Our results suggest that, even when surface area per unit mass is taken into account PLA is more sorptive to Cd than PE. This may have implications for Cd bioavailability in Cd-bearing soils where PE mulches have been replaced by PLA mulches. Further, our results suggest that as MPs weather and biofilms develop on them, Cd mobility will be further decreased.

### **3.21.P-We265 A recipe for plastic: Expert insights on plastic additives in the marine environment**

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The production and consumption of plastic products had been steadily increasing over the years, leading to more plastic waste entering the environment. Plastic pollution is ubiquitous and comes in many types and forms. To enhance or modify their properties, chemical additives are added to plastic items during manufacturing. The presence and leakage of these additives, from managed and mismanaged plastic waste, into the environment are of growing concern. In this study, we gauged, via an online questionnaire, expert knowledge on the use, characteristics, monitoring and risks of plastic additives to the marine environment. We analysed the survey results against actual data to identify and prioritise risks and gaps. Participants also highlighted key factors for future consideration, including gaining a deeper understanding of the use and types of plastic additives, how they leach throughout the entire lifecycle, their toxicity, and the safety of alternative options. More extensive chemical regulation and an evaluation of the essentiality of their use should also be considered.

### **3.21.P-We266 Low toxicity of environmental plastic from aquatic and terrestrial habitats**

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The LABPLAS H2020 developed a Plastic Toxicity Testing Scheme for assessing the impact of plastics and associated chemicals on the environment. With that aim, we adapted internationally accepted ecotoxicological methods to assess plastic toxicity using a battery of bioassays representative of aquatic (both freshwater and marine) and terrestrial habitats. The scheme is based in recent consensus on environmental fate and effects of plastics: i. plastic litter fragments in the environment as a consequence of weathering producing secondary microplastics. ii. the smaller the particles the higher impact on environmental health. iii. chemicals intentionally or unintentionally added to polymers are the main toxicological concern. Field plastic litter sampling was conducted in 2022 and 2023 in the North Sea, Thames and Elbe river basins, Baltic coast, and a river basin in NW Iberian Peninsula that includes a drinking water reservoir and agriculture land. Plastics were ground down to <250µm particles. Leachates were obtained according to a standard methods (Almeda et al. 2023), using a 10 g/L solid-to-liquid ratio, and organisms were exposed to serial dilutions of the leachates (Tier I) and plastic particles of ingestible size range (Tier II).

This methodology is suitable to test the environmental toxicity of any plastic disregarding size or origin, either environmental samples or samples of commercial plastic materials. When this scheme was applied to monitor ecotoxicity of plastic litter across Europe, moderate to nule toxicity was observed in aquatic tests, and in those cases where toxicity was reported individual items were identified as responsible for the negative effects. These include cigarette butts and electric cables. Otherwise, weathered plastic litter does not seem to pose a relevant environmental risk to microfauna or flora in aquatic ecosystems. In contrast, plastic litter of terrestrial origin inhibited earthworm reproduction according to a dose:response pattern, pointing at potential ecologically relevant effects on these key organisms in soil communities.

### **3.21.P-We267 Comparison of Species Sensitivity Distribution Methods for Risk Assessment of Microplastics**

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Most papers published on ecological risks of microplastics (MP) are based on laboratory studies with limited exposure profiles or field studies that focus on the occurrence of MPs in aquatic organisms. However, the current scientific literature and knowledge regarding MP toxicity is sparse and often challenging to interpret, which can introduce uncertainty when conducting risk assessments (RAs). Most MP RAs currently published use a species sensitivity distributions (SSDs) approach. SSDs are a commonly used method that compare toxicity across species by taking a single point (e.g., NOEC or EC50) from a dose response analysis, however this approach can also underscore the variability and uncertainty in the data. In the past decade, there has been an increase in research and regulatory activity directed at supporting MP RAs that utilize the SSD approach. For example, in 2018 the state of California passed legislation requiring a comprehensive literature review to determine if a RA could be conducted for both human health (via drinking water) and aquatic organisms. California concluded there was insufficient data to conduct a formal RA for human health, but sufficient data were available to generate SSDs based on no observed effect concentrations (NOECs) or lowest observed effect concentrations (LOECs). The SSDs were used to define a 5 percent hazard concentration of 5 particles/liter which is cited in the draft 2024 California Integrated Report: Surface Water Quality Assessments as the threshold used to determine listings waterbodies based on MPs. A refinement to the SSD approach is to use the full dose-response curve (DRC) from each study and integrate these curves into one composite DRC. This approach was first developed by USEPA's Office of Pesticide Programs to support ecological RAs of pesticides, and subsequently applied to site assessments of legacy contaminants (e.g., PCBs). This method produces a composite DRC and confidence interval that reflects the variability and uncertainty in the DRC for each study. Here we compare the threshold values for MPs when SSDs are constructed using the NOEC/LOEC approach and the DRC approach applied to the same dataset. We find that when all study treatments are incorporated into the composite DRC the resulting confidence intervals span many magnitudes more than the SSD approach, this highlights the extent to which the methods used in RA reflect uncertainty which needs to be considered when conducting RAs.

### **3.21.P-We268 Bioassay-based hazard assessment of chemical mixtures released from plastics: PlastChemTox evidence map**

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Throughout their life cycle, plastic materials and products can release diverse chemicals, many of which are known to be hazardous for human health and/or the environment, while others remain to be characterized for their hazards or even identity, as both can often be unknown. Inventories of plastic chemicals are being constantly extended, the most recent being the PlastChem database, where we compiled over 16'000 known plastic chemicals. Despite the growing knowledge, chemical analytics-based individual assessment of all substances released from plastics remains unfeasible. Instead, assessment by means of bioassays, also referred to as effect-based testing, could provide an alternative approach for initial hazard assessment of chemicals in plastic leachates. To review the state-of-the-science in this growing field, we performed systematic evidence mapping of the scientific literature, focused on the question “Which types of toxicities or bioactivities have been measured for chemical mixtures released from which types of plastic materials or products?” A literature search done in March 2023 identified 5874 records, for which the title/abstract were then screened using the following criteria: Population – plastic materials or products; Intervention – release of chemicals from plastic materials or products in any type of medium; Outcome – toxicity or bioactivity testing of thus obtained chemical mixtures, regardless of the results obtained. For the 288 records that remained after the first screening, full texts were searched. Relevant data could be extracted from ca. 200 records. Preliminary results show that bioassay-based testing could offer a rapid and efficient assessment of potential hazards. However, currently available studies were found to be highly diverse, spanning a large range of arbitrary choices in terms of the types of polymers tested and bioassays applied. Future research should seek to (i) establish a guidance for rational selection of test samples that ensures a more systematic representation of various polymers and product types; (ii) develop more standardized methods for obtaining relevant chemical mixtures; (iii) establish a panel of relevant bioassays covering typical hazards that can be expected, taking into account the potential use and the likely exposure routes; and (iv) agree on the approaches to interpreting the bioassay results and performing follow-up testing or initiating risk management actions for positive cases identified.

### **3.21.P-We269 Towards risk assessment strategies for nano and microplastic particles**

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The risk assessment of nano and microplastic particles is challenging, due to high uncertainties on release, exposure as well as hazard. The field is characterised by many data gaps, and is quite diverse in the sense that nano and microplastic encompass a huge variety in different polymer materials, size fractions, as well as properties that are tailored by the addition of various additive chemicals.

To facilitate risk assessment, within the Horizon 2020 project PlasticsFatE two different approaches are developed with different scopes, namely decision trees to support Integrated Approaches to Testing and Assessment (IATAs) as well as a Prospective Multi Criteria Decision Support System (PMCDs).

IATAs provide a structured and hypothesis-driven approach to the toxicity testing of nano and microplastic particles including plastic-associated chemicals. It basically gathers all available information on impact and interaction of a substance with organisms, and for this purpose all science-based data, information and knowledge is integrated (e.g. phys.-chem. properties of NMP, appropriate test methods, in vitro, in vivo ...). The current focus of our work is on oral exposure via food contact materials, and to support the development of IATAs, decision trees are generated that consider all available knowledge for that specific route of exposure.

PMCDs, on the other hand, is a tool for early risk assessment of plastics for specific applications and is applicable in early developmental stages. It considers polymers, their basic phys.-chem properties, additives, material fate and adsorbed contaminants. Based on these prospective risk indicators and their uncertainties it allows the identification of risk minimized materials. PMCDs is designed to deal with data gaps and uncertainties. If not enough data is available, the PMCDs falls back on a set of already implemented typical values obtained from modeling, databases and published previous results of experiments. In addition to quantitative input by the user, the processing of qualitative information is also supported.

Here we aim to present our achievements and developments in the PlasticsFatE project. The two approaches will be compared according to scope, specificity and applicability for various interest groups, as well as data needs.

### **3.21.P-We270 Towards safe circular economy by assessing chemical risks of plastic recycling**

**Salla Kristiina Selonen, Päivi Fjäder, Johanna Järvisistö, Sari Kauppi, Matti T. Leppänen, Hanna Niemikoski, Noora Perkola and Helena Dahlbo, Finnish Environment Institute (SYKE), Finland**

The production of plastics has increased explosively. Although the history of plastics is relatively short, more than 390 million tons of plastics were produced in 2021 and it has been estimated that the production will double during the next 20 years. Almost half of the plastic produced is used in single use products like packages. To save the non-renewable natural resources, it is essential that this material after used is recycled as efficiently as possible. Waste Framework Directive 2008/98/EC and Directive 94/62/EC on packaging and packaging waste, have set the ambitious goal that 50% of plastic packaging should be recycled in 2025. In addition to plastic packages, plastics from other uses with large volume should also be covered by recycling as well. These include plastics from building and construction, automotive, households, agriculture as well as the plastics from electronics and electrical devices.

However, concerns on the chemical safety of the recycled plastics have been raised. The recent report of the United Nations Environment Programme stated that over 13 000 different chemicals have been identified in plastic materials, from which over 3 000 were identified as substances of potential concern. To ensure the chemical safety of the recycled plastic materials, the materials containing hazardous chemicals should be identified and removed from the material cycle.

In this presentation, the subproject “Towards safe circular economy by assessing chemical risks of plastic recycling” of EU LIFE funded project PlastLIFE SIP will be introduced. It aims at safer circular economy of plastics by 1) identification of hazardous chemicals in plastic waste streams, by 2) producing data on the mixture effects of the chemicals in plastics to be utilized in risk assessment, by 3) developing methods for risk assessment of plastics and by 4) creating a framework for assessment of chemical risks of plastic waste fractions. PlastLIFE is an extensive project implementing the national program, Plastics Roadmap for Finland. The PlastLIFE project is co-funded by the European Union (LIFE21-IPE-FI-PlastLIFE). Views expressed in this presentation are however those of the authors only and do not necessarily reflect those of the European Union or CINEA. Neither the European Union nor the granting authority can be held responsible for them.

### 3.21.PC Polymers and Their Chemicals: Environmental Fate, Hazards, and Risk Assessment

### 3.22 The Fate and Effects of Micro- And Nano-Plastics in Relation to Ecosystems

#### 3.22.T-01 Systematic comparison of environmental stresses (shear, humidity, UV, pH, temperature, enzymes) on microplastic fragmentation and release of nanoplastics and dissolved organics.

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In this study, we generated a large experimental dataset to understand the mechanisms for microplastic fragmentation and degradation of their physico-chemical characteristics, as dependent on the resistance to environmental stresses. We selected LDPE, PP, HIPS, TPU, PET, PLA, and PA to cover the diversity of relevant plastics, covering both conventional and degradable plastics. The systematic comparison utilised standardised equipment to apply the stresses that are also combined to represent different ecosystems:

- OECD TG111 (hydrolysis) conditions were applied to test pH, temperature, marine water influence
- ISO 4892 (UV) conditions were applied and varied to test UV, temperature, humidity influence.
- Shear forces varied between the very effective sampling by high-shear sonication (ISO 22293), and lower shear by collision with suspended sand. Additionally, shear forces were applied with measured torque on macroscopic specimen.
- Specific enzymes were applied.

To quantify fragmentation and degradation multiple analytics are utilized. An adaption of the NanoRelease protocol, ISO22293:2020, is used to quantify the smallest fragments, from 0.01  $\mu\text{m}$ , thus allowing to show micro- to nano-plastic fragmentation. Chemical degradation is assessed via ATR-FTIR, DSC and GPC analysis, as well as microcracking via SEM. Dissolved organics (DOC) are quantified after filtration at 0.02 $\mu\text{m}$ .

As expected, microplastic degradation depended on the polymer type and specific environmental conditions. DOC are utilized to ensure mass balance by quantifying any dissolved components, and turned out to be a significant mass transfer for irradiated LDPE and HIPS, and even the dominant mass transfer for PA-6 and TPU. In contrast, the PP with UV-stabilizing additives remained stable with no significant releases, as expected. Hydrolytic & enzymatic degradation and fragmentation. Hydrolysis under OECD TG 111 conditions up to 1 year was compared to marine water and enzyme-containing media. The positive control PLA depolymerised at 65°C to DOC, confirmed as lactic acid. All other polymers showed more subtle degradation, e.g. the molar mass of the PA microplastics reduced up to a factor 0.5, releasing also DOC, but few fragments. The combined results on shear forces allow a scaling of the influence of forces on the ablation and abrasion of fragments from aged microplastic. The generated data is also used for the parameterization of the new fragmentation model FRAGMENT-MNP.

#### 3.22.T-02 Impact of Microplastics and Additives in Marine Ecosystems

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Plastic pollution poses a complex threat, not only due to its physical presence but also to the inclusion of chemical additives during production. The increasing amount of microplastics (MP) and their associated chemicals resulting from fragmentation is an intricate yet poorly understood hazard to marine ecosystems. Most toxicity assessments of MP rely on pristine reference materials that inadequately represent environmental particles from everyday consumer products, leading to gaps in understanding their impact. This study aimed to discern the role of MP as a pollutant and a carrier of chemical additives while distinguishing between long-term effects originating from MP versus those from additives in marine species. Fifty plastic products were analyzed, covering various polymers and additive chemical profiles, using non-target chemical screening. Initial baseline toxicity of extracts from each product using Bacterial Luminescence and Algal Growth tests, indicated varied toxicity across products, with higher chemical content correlating with increased toxicity, particularly in elastomer-based products. Comprehensive toxicity testing of leachates from 5 products with high (car tire rubber (CTR), balloons, washing gloves, shoe soles (SS)) or low (PET bottle) baseline toxicity revealed significant impacts on several organisms across trophic levels. Leachate exposures led to impaired fertilization, larval development, and physiological disruptions in copepods, oysters, sea urchins, polychaetes, cod larvae and primary cells from mussels and halibut. Microalgae exposed to leachates from CTR displayed hindered growth and photosynthetic capacity, primarily due to oxidative stress. Juvenile mussels exposed to SS leachates also showed impacted clearance rate and lipid peroxidation. Investigations using cod larvae and adult mussels showed that the additive chemicals in CTR, rather than the particles alone, drove significant adverse effects on survival, hatching success, neurotoxicity and oxidative stress. The complexity of assessing the impact of chemical mixtures associated with plastic consumer products highlights the need to use diverse organisms and endpoints across biological levels to identify key toxicity drivers. This research emphasizes the critical need for mechanistic assessments to discern toxicity drivers between MP leachates and particles and identify key polymer-associated chemicals for potential mitigation strategies to reduce environmental impacts.

### 3.22.T-03 Microplastics-Induced Multigenerational Gut Microbial Changes in *Daphnia magna*: A Matter of Plastics or Particles?

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Although originally described in oceans, microplastic (MP) pollution now spans the globe and all kinds of ecosystems. As MPs are being found throughout the food web, interest in their effects on organismal health is growing. One dimension of health that has been neglected in MPs research is the microbiome. These host-associated consortia of microbes and their genes interact with host immune and nervous systems and play a vital role in host metabolism. Thus, alterations to the microbiome have been implicated in various diseases. While emerging research shows links between MPs and microbial disturbance, potential mechanisms and multigenerational impacts have yet to be described. Here, we investigated whole-organism microbial alterations in response to MPs exposure using an experimental design with both pristine as well as wastewater-treated (wwMP) polystyrene (PS) MPs (< 63 nm) in three different particle concentrations (80, 400, 2,000 particles mL<sup>-1</sup>), plus two different control types (low and high food levels). All this was applied across four generations of *Daphnia magna* individuals, a staple model organism in ecotoxicology. The majority of MP (either pristine or wwMP, we found no significant differences between them) effects on the daphnid microbiome (alpha diversity, beta diversity in terms of composition and heterogeneity) can be explained as particle effects, since the treatment with increasing MP concentrations mirrors the treatment with high food (i.e., high algae concentration) vs low food availability. This coupled with the increase in alpha diversity in higher MP concentration groups lends support to the hypothesis that MPs and other particles act as vectors for microbes to gain access to host-associated microbiomes. We observed a generational effect in that the effects of particles on the microbiome grew over the generations (from F0 to F3). Despite this, there were still MP-specific effects, especially on more rare microbial components of the microbiome. Although daphnid microbiomes treated with higher MP concentrations mirrored those in the presence of only high food levels, the MP-treated daphnids were in no way as healthy, as our previously published work shows lower survival and reproduction rates in higher MP concentration groups. This work shows the importance of choosing a representative control group when investigating MP effects and warns against equating a lack of changes to the microbiome with a lack of negative host health impacts.

### 3.22.T-04 Swimming in plastics: How nanoplastics disrupt larval fish neurobehavioral and molecular rhythms?

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Nanoplastics (NPs) have attracted global attention due to their potential ecological and health impacts. Previous ecotoxicological studies have reported nanoplastic toxicity in various aquatic organisms but there is still limited understanding of how and why different nanoplastics affect or do not affect embryonic development and early life stage behaviors. Here, we investigated the embryonic and neurobehavioral effects of polystyrene nanoplastics (PS-NPs) with different sizes (0.05  $\mu\text{m}$  to 1  $\mu\text{m}$ ) and surface charges (plain PS-NP, amino-modified PS-NP, and carboxyl-modified PS-NP) on early-stage zebrafish. Zebrafish embryos were exposed to PS-NPs at a wide range of concentrations (0.1 to 10 ppm) from 2 to 120 h postfertilization. The dose-responsive toxicological assessment included mortality, hatching, deformities, locomotor activities (distance, rotation, and mobile state), as well as biochemical and global transcriptomic responses. The results showed that nanoplastic exposures did not affect the mortality and hatching rate of zebrafish but caused morphological deformities and significantly disrupted the swimming behavior of larvae. The 3D high-resolution bioimaging confirmed the accumulation of PS-NPs in the larvae. Furthermore, RNA-Seq and bioinformatical analyses predicted and identified the key biological processes and molecular pathways significantly affected by PS-NPs, such as regulation of circadian rhythm, response to light stimulus, photoperiodism, and circadian rhythm. These findings provide new insights into the surface-charge-dependent sublethal neurotoxicity of nanoplastics and highlight the challenge of assessing the ecological risks of nanoplastics due to the high complexity of nanoplastic mixtures in the aquatic environment.

### 3.22.P The Fate and Effects of Micro- And Nano-Plastics in Relation to Ecosystems

#### 3.22.P-Tu309 Monitoring of microplastics in 132 Iowa Lakes in relation to recreational activities and land use

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Global annual plastic production rate is approaching 400 million metric tons, with substantial amounts invading aquatic environments yearly. Numerous studies have been conducted monitoring marine environment plastic litter. However, an understanding of the magnitude of plastic litter in freshwater ecosystems is lagging, particularly for microplastics (MP (s) - 100 nm to 5 mm in length/diameter). Their ubiquitous presence and small sizes are potentially concerning, since MP effects remain inadequately understood. Our objective was to document the concentration of MPs in lake surface waters, and investigate how abiotic, biotic, and anthropogenic elements explain the variability of MP concentrations among lakes. We sampled MPs in 132 Iowa lakes, collected throughout the water column with a Wisconsin net (mesh sizes of 63  $\mu\text{m}$ ). A fully automated custom-built Bruker LUMOS-II Fourier-transform infrared (FTIR) spectro-microscope was employed to identify MPs. Average MP concentration across lakes was 5.2 particles/L, with dimensions averaging 139  $\mu\text{m}$  in length and 77  $\mu\text{m}$  in width. Predominant MP polymers collected were polyvinyl chloride (46% of all MPs), polyester (30%), and polyethylene (11%). To explore the variables that best described the variability in MP concentration among lakes, two variance partitioning analysis (VPA) models were created: one classical and one based on Louvain Groups. The classical model explained 7.5% of data variability based on: roads; developed medium, and high intensity land cover; sewage plants; and thermocline. The second model explained 8.3% of data variability based on: lake area; max lake depth; zooplankton tow depth; Secchi depth; lake perimeter; lake shoreline development factor; evergreen forest cover; and thermocline. Overall, the presence of roads, developed low, medium, and high intensity cover were positively correlated with MP concentration, alongside household visits standardized to the lake area. Lake area, Secchi depth, lake perimeter, lake shoreline development factor, lake maximum depth, and zooplankton tow depth were negatively correlated with MP concentration. The presence of thermocline was negatively associated with MP concentration. MPs currently do not pose a significant threat to Iowa lakes, based on the modeled hazardous concentration affecting 5% of species, however, continuous monitoring is advised.

#### 3.22.P-Tu310 Understanding the Seasonal Distribution of Microplastics Along Two Major European Rivers: River Elbe (Germany) and River Thames (UK)

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The ubiquitous presence of microplastics (MPs) in the environment is well-known, with research on freshwater systems growing. However, the spatial and seasonal factors affecting microplastic spread and abundance in rivers is not well-studied, despite an understanding that these are likely significant factors influencing microplastic concentrations. This study analysed surface water samples from two major European rivers, the German part of the River Elbe and the River Thames in the UK. Samples were collected using *in situ* filtration, collecting of particles down to 10  $\mu\text{m}$  from hundreds of litres of river water. Microplastics were extracted using digestion and flotation, and Fourier-Transform infrared imaging spectroscopy ( $\mu\text{FTIR}$ ) used to analyse particles > 25  $\mu\text{m}$ . Preliminary data suggest that the microplastic concentrations were highly variable between sites and seasons in both the Thames and the Elbe. While the maximum concentrations in both rivers were detected in summer, some sites showed higher concentrations in winter. The microplastic load in the Thames was generally higher than in the Elbe, with the four highest concentrations in the Thames all higher than the maximum concentration detected in the Elbe. The maximum concentration in the Thames (1761 MPs/m<sup>3</sup>) was more than two times higher than the maximum in the Elbe (837 MPs/m<sup>3</sup>). In contrast to our hypothesis, the highest concentrations of microplastics in the Thames and the Elbe were detected in upstream sites, where urban influence is low. At one of the two estuarine sites in the Elbe very few microplastics were detected in either the summer or winter (6.6 and 1.7 MPs/m<sup>3</sup> respectively), while MP concentrations in the Thames varied at tidal sites depending on the season. Low concentrations in the lower Elbe sites are despite proximity to heavily urbanised locations, highlighting the importance of local hydrography in influencing the microplastic abundance and distribution. These data suggest that microplastic concentrations in rivers are generally higher than estuarine and coastal waters, likely due to the

proximity to land-based inputs, with microplastics becoming dispersed and diluted upon reaching the marine environment. Further analysis will be carried out to interpret any observed differences in plastic loads in between the seasons, rivers and sites. Seasonal samples for 2023, plus data on microplastic abundance in fish from these rivers are currently being processed and will also be included.

### **3.22.P-Tu311 Distribution and characterization of microplastics in the coastal areas of the Mediterranean Sea**

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The production and consumption of plastic have drastically increased over the years, due to its cost-effective, durable, lightweight, and resistant characteristics. The same characteristics determine its high dispersion in marine waters and, consequently, within marine organisms, affecting the abundance and diversity of their microbiota. The Mediterranean Sea, as is now well known, is one of the environments most affected by Microplastic pollution, and for this reason, it is becoming increasingly necessary to implement capillary and constant control and mitigation strategies. This is the context for the MICROPLASMED project, which studied the relationship between the role of the marine microbiome as an indicator of different levels of microplastic pollution. To this end, surface water samples were collected between April and August 2022 in different coastal areas of the Mediterranean Sea from five different FAO divisions, aiming to map the distribution, concentration, and composition of the microplastics present. At the same time, samples of *Mullus barbatus* (red mullet) were collected and their guts were analyzed for comparison with the results obtained from the water samples analysis. The results showed the presence of microplastics in 95 percent of the water samples, where the highest concentrations were found in Ventotene (Italy) and Istanbul (Turkey), confirming a close correlation between the amounts of microplastics and highly populated areas with high rates of tourism, intense ship movement, and industrial, urban and agricultural discharges. Plastic polymers were characterized by micro-FTIR together with a high percentage of cellulose fibers and fragments of polysaccharide origin. The samples' analysis not only allowed us to obtain essential information on the concentration of microplastics along the Mediterranean coasts but also on the importance of a complete characterization through the speciation of the polymers. This aspect is fundamental to understanding their source, estimating the possible impacts on the marine and coastal ecosystem, and assessing their distribution, but above all to distinguishing synthetic from natural and not lead to an overestimation of the final data. The data obtained represent an important contribution not only to the study of the distribution of microplastics but also to the development of common analytical protocols that allow unambiguous methods of analysis and representation of the results obtained.

### **3.22.P-Tu312 Emission characteristics of microplastic in urban stormwater runoff: rainfall characteristics and land-use patterns**

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Stormwater runoff is considered a major pathway for land-based microplastic transportation to aquatic environments. We investigated the emission characteristics and loads of microplastics to aquatic environments through urban stormwater runoff during four rainfall events by applying time-weighted stormwater sampling at stormwater outlets from industrial, residential, and commercial catchments and highways. MPs were widely detected in stormwater samples from industrial (1-2,480 n/L), residential (2-1,080n/L), commercial (3-1,810n/L) catchments, and highways (2-2,650 n/L). During the rainfall event, the amount of MP emitted from industrial, residential, commercial catchments and highways was calculated to be  $1.54-46.1 \times 10^8$ ,  $0.63-28.5 \times 10^8$ ,  $0.63-6.30 \times 10^8$ , and  $0.13-8.73 \times 10^5$  particles, respectively. Polypropylene, polyethylene, and polyester were found as major polymers, accounting for around 70% of total microplastics. The fragment was the dominant shape of microplastics, and the most common size class was 20-100  $\mu\text{m}$  or 100-200  $\mu\text{m}$ . The discharge characteristics of microplastics inter- and intra-event were affected by the land-use type and rainfall characteristics. The microplastics in stormwater were more concentrated when the number of antecedent dry days (ADDs) was higher; the concentration of microplastics generally peaked in the early stage of runoff and varied according to rainfall intensity during a rainfall event. The MP load per drainage area was highest in the industrial catchment. The contamination level and load of microplastics were heavily affected by the total rainfall depth. Most microplastics were transported in the early stage of runoff. The microplastic emission via stormwater runoff was significantly higher (99%) than that through the discharge of wastewater treatment plant effluent (1%) in the same area, implying that stormwater runoff is the dominant pathway for transporting microplastics to aquatic environments.

### **3.22.P-Tu313 Development and Validation of High-Throughput Methods for the Sampling, Extraction and Analysis of Marine Microplastics**

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To capture the full extent and long-term impacts of global microplastic pollution, reliable and harmonized chemical-analytical protocols (sampling and sample preparation) and detection techniques are mandatory. We have analyzed microplastics in subsurface water or sediment samples from the tropical Indian Ocean, the Northeast Atlantic Ocean, the East Greenland coast, and the Congo Canyon using a completely validated sampling (stainless-steel fractionated filtration unit), sample purification (microwave-assisted enzymatic-oxidative digestion) and automated imaging workflow (laser direct infrared (LDIR) imaging). Additionally, complementary mass spectrometry techniques such as ICP-MS/MS or Py-GC-MS were applied for validation



and method comparison purposes. Finally, only the application of a standardized protocol enables direct comparability of the large datasets which is usually strongly hampered in microplastic research. The complex results enable conclusions about the exposure levels as well as possible entry, fragmentation, and transport pathways. We investigated areas with an a priori high anthropogenic plastic footprint and remote areas. However, the results did not always confirm the assumptions and underpinned that the distribution patterns of microplastic particles and fibers still pose scientific puzzles.

### 3.22.P-Tu314 The spatial abundance and distribution of microplastics in the surface sediment of Masan Bay, South Korea

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Microplastic pollution emerged as a growing global environmental issue due to its widespread occurrence and potential ecological repercussions. This study focused on evaluating the presence, characteristics, and potential sources of microplastics in Masan Bay, South Korea. As Masan Bay faces escalating environmental challenges amid industrial and urban development, a thorough analysis of microplastic presence in its sediment became imperative. Masan Bay, one of the special management sea areas in Korea, confronts significant land-based pollution stemming from neighboring international trade ports, industrial complexes, and residential regions. In June 2022, sediment samples from the surface layer up to 3cm depth were collected across 27 stations within Masan Bay using Van Veen grabs. Microplastics were separated from the sediment through density separation employing lithium metatungstate (LMT, 1.6 g/cm<sup>3</sup>) and digested with wet peroxide oxidation. These samples were filtered through silicon filter paper (pore size: 10 µm) and were examined using micro-Fourier transform infrared spectroscopy (µFT-IR). The abundance of microplastics in Masan Bay sediment ranged from 3,190 to 77,600 n/kg d.w. (mean: 19,800±17,100 n/kg d.w.). The dominant shape of microplastics was fragments (> 80%), followed by the fibers (19%). The predominance of low-density polymers such as polypropylene (53%), polyethylene vinyl acetate (10%), and polyethylene (7%) was found in the bottom sediments, which might be due to aggregation with biogenic (or inorganic) matters or fecal pelletization process in the water column. This finding indicates the role of marine sediment as a sink for microplastics. Non-fibrous particle sizes dominantly ranged between 20-100 µm or 100-200 µm, while fibrous particles exhibited a broader range (20-5000 µm). The standing stock of microplastics in the sediment layer up to 3cm in Masan Bay was calculated to be approximately 21.3 trillion particles. The microplastic concentration showed an increasing trend from the outer to inner areas of the bay, and the inner side exhibited higher polymer diversity. These findings indicate a possibility of a substantial inflow of microplastics into the inner areas of Masan Bay from nearby residential and industrial zones and ports. Moreover, these insights provide crucial information about the occurrence and influx of microplastics in Masan Bay.

### 3.22.P-Tu315 Characterization of Plastics Obtained in the OSPAR Monitoring for Marine Litter in 5 Beaches of the Bay of Biscay

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During the last years, the scientific community focused in elucidating the impact of plastic pollution on the marine environment; especially regarding micro and nanoplastics (MNPs). But, in order to achieve this challenging goal, ecotoxicological studies need to move from commercial pristine MNPs to more environmentally relevant ones. In this context, the FIERA project aims to assess the toxicity of a representative mix of environmental plastics collected in the southwest of the Bay of Biscay. As a first step, plastics were collected following the OSPAR guideline for Marine Litter Monitoring (100 m transect) once per season in 5 beaches of the Basque Country: 4 in Spain (Gorrondatxe-Getxo, Alkolea-Mutriku, Orrua-Zumaia and Murgita-Donostia from January to October 2023 and 1 in France (Tarnos-Baiona) from July 2023 to March/April 2024. After each sampling, items per beach and season were divided into 3 categories (foams, fragments and recognizable objects), the colour and weight of each item were annotated and the polymer composition determined by FT-IR. Almost 94% of the items collected in January and July 2023 were plastic polymers. No clear site- or season-dependent trend was observed in the proportions of foams, fragments and recognizable objects. White was the predominant colour followed by transparent in all beaches and seasons, with the only exception of Alkolea. Regarding polymer composition, 9 polymers were found (polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyethylene (PE), polyamide (PA), polyurethane (PUR), ethylene-vinyl acetate (EVA) and polyacrylamide (PAM). PE was the only polymer found in all samplings, while PS and PP were found in all beaches, but not all seasons. Taking into account the number of items, the abundance of polymers was PE > PP > PS > PET > PUR > PVC > PA > EVA = PAM. When the weight of items was considered, abundance was: PE > PVC > PP > PET > PA > PS > PUR > EVA > PAM. Polymer abundances by items or by weight both varied with beaches and seasons. Results highlighted the complexity of characterizing plastic pollution in the Bay of Biscay, due to differences found between different beaches and seasons. \*Funded by the Spanish MICIN project FIERA (PID2021-128600OB-I00, MCIN/AEI/ 10.13039/501100011033 and “ERDF A way of making Europe”) and the Basque Government through a grant to the consolidated research group IT1743-22 and a postdoctoral fellowship to NGS.

### 3.22.P-Tu316 Hemocyte Responses upon Foodborne Exposure to Three Sizes of Polystyrene Nanoplastics on Mussels *Mytilus galloprovincialis*

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The evidence of the harmful effects of microplastics on marine organisms has greatly increased in the last years, but information regarding the impact of nanoplastics (NPs, <1 µm) is still insufficient. Bivalves are widely used sentinel organisms due to their filter-feeding ability, among others. This work aims to elucidate the differential toxicity of 3 sizes of polystyrene (PS) NPs (50, 200 and 1000 nm) on the hemocyte responses and condition index (CI) of mussels *Mytilus galloprovincialis*. Mussels were dietarily exposed for 7 days through microalgae *Isochysis galbana* to a low dose (LD:10<sup>3</sup> NPs/mL) and a high dose (HD: 10<sup>8</sup> NPs/mL for 50 and 200 nm NPs and 10<sup>6</sup> NPs/mL for 1000 nm NPs). Each experiment was performed twice, as follows: E1&E4) 50 nm, E2&E5 200 nm, E3&E6 1000 nm. Despite the 6 experiments were run in less than a month, there were significant differences between the 2 independent experiments of each NP. No differences in CI were found within each experiment, but differences were observed between the 2 independent experiments, possibly due to the spawning events observed during the acclimation period of E3&E4. Similarly, higher reactive oxygen species production was observed in hemocytes of control mussels of E3&E4 compared to E1&E2 and E5&E6, but no differences were seen in NP treatments compared to controls within experiments. Cell viability of control hemocytes decreased in experiments E3&E4 and E5&E6 in comparison to E1&E2. This could have masked the size-dependent response to the NPs, as in E1 hemocytes showed lower viability (LD and HD) and phagocytic activity (HD) than controls, but not in E4. Similarly, hemocytes of mussels exposed to LD in E2 showed lower viability than controls, but the contrary was observed in E5. In conclusion, a trend for size-dependent effects on mussel hemocytes was observed. However, hemocyte responses varied along time, possibly due to the seasonal variations in mussels physiology connected with the gametogenic cycle. Further work is needed to elucidate the impact that MPs and NPs may have in mussels at different periods of their gametogenic cycle to allow their use as sentinel organisms for monitoring MP and NP pollution in the marine environment. \*Work funded by the EC CAS NANOPLASTICS project (DG JRC), the Spanish MICINN FIERA project (PID2021-128600OB-I00, MCIN/AEI/ 10.13039/501100011033 and “ERDF A way of making Europe”) and the Basque Government grant to consolidated research groups (IT1743-22).

### 3.22.P-Tu317 Microplastic uptake and trophic transfer in mid-consumer fish species in a heavily contaminated river

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Multiple urban and sub-urban rivers in the UK were identified to be highly contaminated with microplastics. The River Tame, Greater Manchester, was the most contaminated among these rivers and contained multiple riverbed microplastic hotspots. The majority of microplastic in the River Tame comes from wastewater effluent, and the release of untreated wastewater during low flow is the driving mechanism behind riverbed hotspot formation. The high content of beads and fragments indicates frequent discharge of untreated wastewater, and flooding can effectively entrain the complete assemblages of microplastics and flush them downstream. The river community is therefore subjected to a dynamic microplastic exposure risk, of chronic and acute homogeneous or heterogeneous microplastic assemblages depending on the point source input locations and river flow events. This study aims to determine whether the microplastic in a heavily contaminated urban river (i.e., River Tame) is taken up by biota and transferred across trophic levels. We investigated the occurrence, assemblage, uptake and bioaccumulation of microplastic in the gills and gastrointestinal tract of two mid-consumer fish species (common minnow and three-spined stickleback), as well as the microplastic loads in host fine bed sediments and prey items (invertebrates). A total of 93 individual fish (common minnows,  $n=41$ ; stickleback,  $n=52$ ) were collected from the Reddish Vale and Dukinfield reach of the River Tame, and the gills and gastrointestinal tract ( $n=48$  per organ; minnow = 21, stickleback = 27) were isolated, chemically digested and filtered onto glass-fibre filter. Microplastics were identified and quantified on a digital microscope at 100x base magnification. A melt-test was performed on all suspected particles, and polymer analyses were conducted using  $\mu$ -FTIR spectroscopy. We found that the microplastics in the River Tame spread from the environment into the biota (Mean $\pm$ SEM; Minnow=17.11 $\pm$ 3.93 MPg-1 Ind.; Stickleback=12.39 $\pm$ 1.36 MPg-1 Ind.). Microplastic levels were higher in the population living near wastewater treatment outflow ( $P=0.0016$ ), and fragments (51.7%) and fibres (43.0%) are the most abundant forms of microplastic. Microplastic enters through respiratory and gastrointestinal pathways, and intake in relation to body size varies between species. While bioaccumulation was not observed, we show that there are active cycles of microplastic within the trophic component of the River Tame biota.

### 3.22.P-Tu318 Microplastic accumulation in oysters from Hong Kong's Deep Bay and Yong Shue O.

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In 2016, each person in Hong Kong produced an average of 163.45 kg of plastic waste and about 0.05% of this ends up as marine litter in the form of microplastics (MPs); global trends suggest plastic use has only increased. MPs are plastic particles under 5 mm in size. They are pervasive, being found in over 220 marine species and in almost every oceanic layer. Their small size contributes to their ubiquity in marine organisms, especially filter feeders like oysters. However, lab studies considering MP uptake are often based on exposure to unrealistically high MP concentrations. To better understand realistic MP accumulation in the natural environment, we investigated MPs in sediments, waters, and oysters from two Hong Kong locations of different proximity to anthropogenic activity (Yong Shue O, YSO) and oyster aquaculture (Deep Bay, DB), during the wet and dry season. MPs were extracted by standard potassium hydroxide digestion protocols, followed by filtration onto 47 mm glass-fibre filters of 0.7  $\mu\text{m}$  then 0.1  $\mu\text{m}$  pore size. Quantitative visual selection was conducted at 40x to 100x magnification using stereo- and digital- microscopes. Examples of putative MP will be analysed to ascertain chemical identity. For the dry season, we found that all oyster samples were positive for MP with mean ( $\pm$ SD) abundances of  $82.76 \pm 92.64$  (n=46) putative MP  $\text{g}^{-1}$  of tissue in DB and  $137.1 \pm 161.8$   $\text{g}^{-1}$  of tissue (n=64) in YSO. In DB, the digestive gland contained 48% of our counted MP, the gills had another 28%, and the mantle contained the remaining 24%. These distributions were different in YSO, where most MP was found in the mantle. Our biotic samples were largely made up of transparent fibres (74% of MP) and blue fibres (11% of MP). Other colours and shapes (fragments and beads) were minor contributors. Significantly more MP  $\text{g}^{-1}$  was seen in the YSO than DB location (t-test:  $p=0.042$ ). This difference was not observed in water samples ( $2.78 \pm 1.69$  MP  $\text{L}^{-1}$ , DB;  $1.14 \pm 0.39$  MP  $\text{L}^{-1}$ , YSO; mean  $\pm$  SD, n=3, t-test:  $p=0.179$ ). We now aim to study associations between abiotic and biotic data. Our analysis of the wet season is ongoing but will provide insight into the role of MP inflow from rivers on MP accumulation in coastal oyster populations. Understanding MP prevalence in bivalves has implications for aquatic health and health of humans consuming oysters and our findings may be used to develop effective policy regarding plastic pollution.

### **3.22.P-Tu319 Microplastic Pollution In Biological and Environmental Matrices From An Aquaculture In Portugal And Potential Impacts To Human-Beings**

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Marine bivalves are largely produced in aquacultures for human consumption and can be impacted by several pollutants, including microplastics (MPs), that can cause potential harmful effects. The MP abundance and characterization in surface water, sediments, and bivalve tissues of *Magallana gigas* (diploids and triploids) and *Ruditapes philippinarum* were assessed in an aquaculture at the Ria de Aveiro coastal lagoon (Portugal) and the daily MP intake by humans via bivalve consumption was estimated.

Microplastics in surface water (0.00–5.33 MP particles/L) were mainly transparent fibers (100–2500  $\mu\text{m}$ ) with some seasonal variations, while in sediments (15.47–114.55 MP particles/kg) were predominantly blue and red fragments (100–2500  $\mu\text{m}$ ) with seasonal and tidal variations. Seasonal and tissue differences were observed in *M. gigas* diploid (0.33–5.00 MP particles/tissues), *M. gigas* triploid (0.00–1.00), and *R. philippinarum* (0.00–3.33). Microplastics, mainly pink and transparent films (1–100  $\mu\text{m}$ ), were found essentially in visceral mass, digestive gland, and gills. *M. gigas* diploid and *R. philippinarum* had higher microplastic abundances than *M. gigas* triploid. The main polymers were polypropylene and polyethylene. The daily MP intake via consumption of un-depurated bivalves may pose a threat to human health, particularly to adults, very elderly, and pregnant women.

### **3.22.P-Tu320 STUDY OF THE MICROPLASTIC ABUNDANCE AND COMPOSITION IN GASTROINTESTINAL TRACT OF SEABIRDS FROM THE CANARY ISLANDS DETERMINED BY OPTICAL MICROSCOPY AND ATR-FTIR**

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Currently, plastic pollution is a significant environmental problem worldwide, especially in the marine environment, where plastic debris enters in a wide range of sizes, and particularly the nanoplastics (0.001-0.1  $\mu\text{m}$ ) and microplastics (in the size range of 0.1–5000  $\mu\text{m}$ ) which have a special interest mainly due to their small size, as well as long environmental persistence. In addition, the intake of these micro- and nano-sized plastics by marine animals is the cause of internal injuries, a feeling of satiety and in many cases of death.

The present work is focused on the study of the presence of microplastics in the gastrointestinal tract of Cory's shearwaters (*Collectris borealis*) which are native to the Canary Islands. For this, the dissection of the samples was carried out to isolate the possible plastic particles present in the proventriculus and ventriculus of the birds. The digestion of biogenic organic matter preserving the integrity of synthetic polymer particles is an essential step for the microplastic analyses. Therefore, an appropriate digestion protocol with a base (potassium hydroxide) 70% in combination with an oxidizing agent (hydrogen peroxide) 30% at room temperature during 48 h, were carried out. Different analytical techniques such as optical microscopy and Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR) have been used for detection and identification of the microplastics particles that have been found.

The percentage by weight of microplastics with respect to body weight and stomach weight were 2% and between 3-12%, respectively. Microplastics with a size less than 3 mm were found in the ventricle, while in the proventriculus the size was up to 5 mm. Fibers (34%) and pellets (25%) were the most frequently observed morphology, and, based upon ATR-FTIR analysis, polyethylene (56%) was the most common identified polymer, followed by polypropylene (25%) and poly(vinyl chloride) (19%). This study provides insight into the magnitude of microplastics pollution in the coasts of the Canary Islands and describes a methodology to improve future microplastics studies in Cory's shearwaters (*Collectris borealis*).

### 3.22.P-Tu321 Birds Of Prey and the Threat of Artificial Microparticles

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#### Abstract

Inadequately managed plastic waste results in their accumulation within natural ecosystems. Once in the environment, polymers undergo weathering leading to their breakdown into microplastics (MPs). The increase of MPs contamination has become a serious problem, threatening the environment and organisms, including avian species. Predatory birds occupy the top of the trophic web, and therefore play a vital role in ecosystem balance. This study investigated the presence of MPs and artificial-non-plastic particles (ANPP) in digestive and respiratory systems of four predatory birds, within different environments intertwined with human activity. This included species: Common Buzzard (*Buteo buteo*), Black Kite (*Milvus migrans*), the Eurasian Sparrowhawk (*Accipiter nisus*) and Northern Goshawk (*Accipiter gentilis*). Their digestive and respiratory systems were extracted, chemically digested with KOH and the solutions obtained were filtered through a 25µm stainless steel mesh. Detected microparticles were measured, classified according to their shape (fibers or fragments), quantified and identified using micro-FTIR. The results showed the presence of MPs and ANPPs in the four species, with an average of 7.9 MPs and 9.2 ANPPs per specimen. The predominant shape consisted in small-sized fibers (>98%) of polyester, acrylic materials, and synthetic celluloses. It is important to highlight that all the digestive systems contained at least one MP. For the first time, MPs have been detected in the respiratory systems analyzed of predatory birds. When comparing the incidence of the MPs in digestive and respiratory systems obtained from predatory birds collected in urban areas against rural areas, there was a significant higher presence in the urban areas. These findings underscore the need for further research on the impact of the contamination due to plastics and other anthropogenic materials in predatory birds

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### 3.22.P-Tu322 Concentration, Characteristics and Risk Assessment of Microplastics in Echinoderms of the Western Cape, South Africa: a need for Science-Based Solutions to Mitigate Impacts of Microplastics

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Information and knowledge about microplastics (MPs) in echinoderms inhabiting coastal environments along South Africa is poor. The aim of this study was to determine coastal MP abundance (MPs/I) and concentration (MPs/g) ingested by various echinoderms species. Sampling took place in summer 2020 during low tide at 14 sites, along the coast of the Western Cape, South Africa. MPs were extracted and analysed based on shape, colour, size and polymer type (using an FTIR-ATR). An ecological risk assessment was done to assess the potential risks posed by MPs in echinoderms. MP abundance and concentration was highest in echinoderms sampled from Kalk Bay ( $59.95 \pm 4.68$  MPs/I and  $2.90 \pm 0.38$  MPs/g respectively), identifying stormwater outfall pipes and human activities as the main source of MP contamination. Suspension/deposit-feeding sea cucumbers displayed a high MP abundance ( $17.57 \pm 2.34$  MPs/I) and predator feeding sea stars and cushion stars had high MP concentrations ( $2.47 \pm 0.23$  MPs/g). Suggesting non-selective suspension/deposit-feeding invertebrates ingest more MP particles than other feeding strategies. Filaments were the most dominant MP shape (94.48%) for all echinoderm samples, with black/grey particles being the dominant colour (45.48%). The dominant sizes ingested by echinoderms were 1000 – 2000 µm (31.86%), 2000 – 5000 µm (25.86%) and > 5000 µm (20.24%). Polyethylene (PET) (49.48%) was the dominant polymer type recorded in all echinoderm samples. Based on the risk assessment, MPs recorded at Mouille Point (site 6) poses the greatest ecological risk associated with polymers. MP concentrations reported in this study provides a baseline for future studies along the Western Cape coastline, South Africa. Furthermore, substantive efforts are required to mitigate the impacts of MPs.

### 3.22.P-Tu323 A Plastic Trap? Factors Influencing Microplastics Trapping in Coastal Vegetated Canopies

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Coastal areas are prone to plastic accumulation due to their proximity to land based sources. Coastal vegetated habitats (e.g., seagrasses, saltmarshes, mangroves) provide a myriad of ecosystem functions, such as erosion protection, habitat refuge and nursery grounds, and carbon storage. The biological and physical factors that underlie these functions may provide an additional benefit: trapping of marine microplastics. While microplastics occurrence in coastal vegetated sediments is well documented, there is conflicting evidence on whether the presence of vegetation enhances microplastics trapping relative to bare sites. Moreover, the factors that influence the likelihood of microplastic trapping remain understudied. We aimed to investigate how vegetation structure and diversity as well as microplastic type influences microplastic accumulation in a UK saltmarsh. In September 2022, we sampled sediment from Blakeney National Nature Reserve. Quadrats (N = 20; n = 5) were placed across four levels of species diversity – no vegetation, monospecific grass (*Spartina anglica*), monospecific branched (*Atriplex portucaloides*), and diverse (>3 species). We recorded species presence, percent vegetation cover, and canopy height. In the laboratory, we will isolate, quantify, and characterise microplastics using an overnight organic matter digestion, followed by centrifugal density separation, visual microscopy, and micro-FTIR spectroscopy analysis. We will compare microplastics abundance and characteristics across each level and type of vegetation cover. We predict that (i) sites with high species diversity will have higher microplastic loads than those with low (or no) species diversity; (ii) more structurally complex vegetation will have higher microplastic loads than less structurally complex vegetation; and (iii) different microplastic types will have different depositional patterns. The outcome of this work will enrich our understanding of coastal vegetation as a microplastics sink and inform where hotspots of microplastic accumulation are most likely to occur within a biogenic canopy. By increasing our understanding of microplastics pollution within highly valued coastal habitats, we can aid protection, restoration, and potential clean-up efforts.

### 3.22.P-Tu324 Unveiling human exposure to microplastics from water sources

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This study addresses the growing concern of microplastic (MPs) pollution in water sources through a comparative analysis of MPs presence in tap and bottled water in Spain. The primary goal is to assess and compare MPs concentrations in these crucial drinking water sources. Tap water samples were collected from 24 points across 8 locations, using steel filters with a 25 µm opening size. Simultaneously, bottled water assessments focused on 1.5 L poly(ethylene terephthalate) bottles from major Spanish brands. The methodology involved particle collection using 0.8 and 1 µm pore size filters, followed by photographic, spectroscopic, and micro-FTIR identification. In tap water, the average concentration of MPs was  $12.5 \pm 4.9$  MPs/m<sup>3</sup>, accompanied by anthropogenic particles (APs) at  $32.2 \pm 12.5$  APs/m<sup>3</sup>, with polyamide, polyester, and polypropylene identified as the main polymers. In contrast, bottled water showed an average MP concentration of  $0.890 \pm 0.192$  MPs/L, primarily composed of white and transparent polyester particles, accompanied by artificial nonplastic particles (ANPPs) at  $1.73 \pm 0.32$  ANPPs/L primarily composed of cellulose. Daily intake estimates ranged from 12 to 55 ng kg<sup>-1</sup> day<sup>-1</sup>, suggesting a low-risk scenario. The results indicate that, although MPs in tap water present minimal health risks, bottled water, while still low-risk, exhibits notably higher concentrations of plastic. This highlights the importance of considering both sources in thorough assessments. The study contributes significantly to our understanding of microplastic exposure, emphasizing the necessity for additional research and the adoption of sustainable water management practices. The relevance of this research lies in advancing our knowledge of microplastic contamination across various water sources, offering insights that can inform the development of public health policies and practices. The comparative approach of the study enhances its applicability, providing valuable guidance for future efforts aimed at mitigating the impact of MPs on both human health and the environment.

### 3.22.P-Tu325 Priorities to Inform Microplastics Management, Monitoring, and Research: A California Case Study

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The state of California, USA, proposes to establish the first statewide microplastics monitoring program designed to inform management decisions to protect aquatic ecosystems from microplastics pollution. However, scientific understanding of microplastics (including tire-wear particles) presence, fate, and impacts lag behind the urgent information needs environmental policy-makers have to manage microplastics pollution. Therefore, it is critically important for microplastic researchers to understand the priority “management questions” policy-makers and water quality managers have to inform near and long-term environmental policy decisions, and prioritize research funding and efforts to address these “management questions” and associated monitoring data needs. We gathered input from federal, state (California), and local government agencies, and local water quality managers to understand the priority management questions and information needs on microplastics. We synthesized diverse input into a comprehensive framework, and articulate clear monitoring goals and objectives for a statewide monitoring program. We also identify priority research needs to address priority management questions, and invite SETAC members to provide input as well through an on-line questionnaire. Input received during SETAC will be incorporated into the developing California statewide microplastics monitoring strategy. Lessons learned from California’s approach may be applicable widely to other governments embarking on developing monitoring strategies to inform microplastics management.

### 3.22.P-Tu326 Incorporation of Micro- and Nanoplastics in Sea Ice During Freezing

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Despite being remote, the Arctic is widely contaminated by microplastics (MPs, 1µm to 5mm). In fact, concentrations of MPs >10mm are orders of magnitude higher in sea ice compared to the underlying seawater. This could put the polar food web at high risk of exposure since the porosity of sea ice, generated by brine channels, is an important habitat for photosynthetic organisms. Furthermore, the environmental fate of small MPs (1µm to 10mm) and nanoplastics (NPs, <1µm) at the seawater/sea ice interface is unknown due to the analytical challenges involved in quantifying them. Therefore, it is crucial to understand how MPs and NPs become enriched in sea ice. To achieve this, we developed a novel laboratory experimental set-up which mimics sea ice growth and allows us to quantify plastics within the ice and brine channels separately. Our hypothesis was that the extent of particle incorporation into sea ice is dictated by their size and density. To this end, we compared the fate of various species: a dissolved molecule (Rose Bengal), NPs, nanosoot, and two MPs of similar size but different densities. Rose Bengal and nanosoot were quantified by absorbance spectroscopy. Model metal-doped NPs and MPs were quantified by inductively coupled plasma mass spectrometry using trace elements as a proxy for plastics. Artificial sea ice was generated with a temperature gradient of 1.0 to -5.1°C. At the end of the experiment, the ice was centrifugated to remove the brine. The morphology of the ice (assessed by m-computed tomography) and its salinity was similar to that of young natural ice. For all treatments, we quantified all species in brine, ice and the underlying liquid. Salts and Rose Bengal were equally enriched in the brine channels and depleted from the ice. However, MPs followed divergent transport pathways depending on their density. High-density MPs were depleted in both brine and ice whereas low-density MPs were slightly depleted from brine and enriched in ice. While NPs and nanosoot were depleted from ice, their rate of enrichment in brine was intermediate between the one of dissolved species and PET-MPs. This suggests that these nanometric particles follow a distinct transport pathway due to their colloidal properties. In conclusion, we can expect the accumulation of low-density MPs in the solid ice matrix and of NPs in the brine channels. This work therefore elucidates which organisms are at risk, and which type of plastic particle they may be exposed to.

### 3.22.P-Tu327 Modification of a Nile Red Staining Method for Microplastic Detection in Environmental Media

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Numerous methods have been developed for microplastics isolation and quantification in various environmental media, many of which require elaborate/expensive analytical equipment and decontaminated lab space. This study seeks to create a reproducible and economical method for the isolation of microplastics in surface water and sediment samples that is reliant on Nile Red staining. We use a Nile Red pre-staining step prior to sample digestion, density separation, and filtration to mitigate downstream in-lab contamination. To test the method generated, we seasonally collected replicate surface water samples from urban streams in the Chesapeake Bay Watershed, USA for one year and quantified microplastic concentrations via fluorescent microscopy. We investigated spatial and temporal microplastic concentrations with the goal of estimating the impact the University of Maryland College Park campus has on microplastic abundance. This method was also applied to sediment samples from the abyssal plain of the Pacific Ocean to see if the method is amenable to the quantification of samples containing substantial and variable sediment loads. The proposed sampling and quantification method found some success in both surface water and marine sediment samples with specific microplastics ( $\geq 20 \mu\text{m}$ ) able to be enumerated.

### 3.22.P-Tu328 Reproducibility and Environmental Relevance: An Impossible Match for Plastic Particles?

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Increased awareness of the potential impact of nanoplastics (NPLs) on biota stems from their higher surface area-to-volume ratios, which impart greater reactivity and adsorption capacity compared to their larger microsized counterparts. The research in this area has progressed rapidly. Nevertheless, the generated data may possess limited relevance due to the trade-off between achieving reproducibility under controlled laboratory conditions and maintaining environmental significance, creating a potential disconnect.

The nanoplastics used in laboratory settings often lack significant analogies with those present or expected in the natural environment, further widening the disparity between laboratory findings and real-world situations. This work aims to underscore the intricacies and potential misunderstandings frequently encountered in published research concerning the size and shape of NPLs.

Current research on nanoplastics appears guided by commercial particle availability and technological constraints (e.g., characterization possibilities), as well as the goal of assigning specific size and shape effects. Despite the relevance of these findings, particularly for industry, the current state of contamination makes the relevance of this information limited to assess the impact of particles already present in the environment, which include different sizes, shapes, ages and additives/contaminants. Therefore, at an initial stage, these restrictions can be overcome by resorting to more complex studies that involve the combination of different sizes and shapes of plastic polymers that would more effectively replicate the varied nature of nanoplastic pollution in the environment. However, it should be noted that such an approach would increase the complexity of research efforts. An initial strategy, recognizing the variability of size and shape proportions between sites and

the current impracticality of on-site characterization, involves employing mixture models for various contaminants. This involves the selection of sensitive species and parameters.

### **3.22.P-Tu329 PlasticFADE: Introducing a Mechanistic Model for Plastic Fragmentation and Degradation in the Environment**

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Plastics are generally durable, yet they fragment and degrade under natural conditions, affecting their mass, residence time, and size distribution in the environment. Existing models that address plastic fragmentation and degradation have provided an oversimplified picture. These models often assume constant rates over time, apply identical rates to different polymers and compartments, and consider inadequate shapes and sizes of plastics. To address this challenge, we are developing a model to calculate specific plastic fragmentation and degradation rates by combining a mechanistic understanding of these processes with statistical techniques. The model accounts for the major influencing factors of fragmentation and degradation: properties of the plastics and conditions of their surrounding environment. Although fragmentation and degradation occur simultaneously under various sources of environmental stress, they are modeled separately in this work. Quantitative data from recent plastic weathering studies have been collected, which are used to derive model parameters and validate the modeling results later. These data have been reported in both mass- and number-based formats, for example, loss of the total mass and particles produced per pellet. Accordingly, a unit conversion approach is proposed for the transformation between mass- and number-based rates. Preliminary results highlight the influence of size, shape, and polymer type on the magnitude and variation of fragmentation and degradation rates. The rates computed by the PlasticFADE (Plastic Fragmentation And Degradation in the Environment) model will have a unit of percentage of weight loss per day. These rates can be used in the calibration of existing plastic fate models and the estimation of global plastic mass budget. In addition, these rates will be integrated – in an upcoming study – into the characterization factors for plastic emissions in life cycle impact assessment (LCIA). The life cycle assessment (LCA) community is now working on incorporating plastic impacts into the LCIA framework. The integration of fragmentation and degradation rates will establish the missing link between the impacts of macroplastic emissions and the impacts of secondary micro- and nanoplastics that break down from the macro-sized debris.

### **3.22.P-Tu330 Relation between Surface Hardness and Elasticity to Carbonyl Indexes of Plastic Products and Environmental Plastic Debris**

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The majority of plastics degrade and break down into macro and microplastics in the marine environment. However, the degradation and fragmentation of plastics in the environment remain insufficiently understood. Particularly, only few studies have reported the physical properties of weathered plastics in the open sea, although properties such as the carbonyl index using infrared spectra have been discussed.

The objective of this study is to uncover the relationship between surface hardness, elasticity and the carbonyl index of micro and macro plastics. Additionally, we aim to investigate the connection between product plastics and environmental plastics. Fifty polypropylene (PP) products were purchased from stores or online shops, and thirty PP films were collected from the open sea in the Pacific and Indian Oceans through collaboration with volunteer commercial ships. Additionally, fifty PP debris items, such as lids from plastic bottles, films, and fragments, were collected from Japanese beaches and rocky shores. A Dynamic Ultra Micro Hardness Tester (Shimadzu) was utilized to measure Martens hardness, and elasticity using a measurement method that we developed. Micro-Fourier Transform Infrared Spectroscopy was employed to identify polymers through ATR on their surfaces and to calculate their carbonyl indexes. The poster will demonstrate the relationship between surface hardness and elasticity and the carbonyl index of weathered PP debris and commercial products.

### **3.22.P-Tu331 Assessing Microplastic Generation in Rivers by Abrasion Experiments**

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Rivers are complex ecosystems in which different flow conditions can affect the fate of plastic materials. Fragmentation of plastic debris into secondary microplastic (MP) and nanoplastic (NP) increases the bioavailability and risk potential for aquatic organisms by the change of size, shape and transport behaviour. Abrasion, as one mechanism of fragmentation, might occur at the river bottom due to mechanical interaction of plastic material and the river bottom. Therefore, the generation of MP from the high density plastics polyamide, polyethylene terephthalate, and polystyrene by abrasion was investigated for different roughness and power input in laboratory experiments. A Taber Abraser 5131 equipped with different abrasive wheels was used to simulate the interaction of plastic materials and the river bottom under wet conditions. The released MP mass was determined and the particles were analysed regarding their particle size distribution and shape.

Our results indicates a relatively small size ( $d_{50}$ ) of the released particles of 40  $\mu\text{m}$  independently of the polymer type and the roughness of sanding paper. However, up to 2 % of the generated particles were in the sub-micron range and can be considered as NP. Thus, the small particle size of released MP would increase the bioavailability of plastic materials. Subsequently, further fragmentation of MP to NP could occur by biological mechanisms or chemical degradation. Nevertheless, abrasion of plastic objects contributes only to a small extent to the formation of NP in riverine ecosystems.

Moreover, we hypothesize that the mass of released MP correlates with the power input and can be described by a linear equation. The parameters controlling the power input of a fluvial system could be derived from empirical equations by Bagnold, Mannig-Strickler or Sipe et al. (2022). Thus, the fate of plastic materials could be predicted in terms of MP and NP formation. In addition, the investigation of their physico-chemical characteristics in combination with abrasion experiments may help to identify polymers, which could generate most secondary MP and MP after release in the environment. Additionally, the knowledge about MP shape is crucial to improve the understanding of the fate of microplastic in river ecosystems. This would contribute to an improved risk assessment of aquatic ecosystems in which high MP release was predicted.

Ref.: Sipe et al. (2022) in Science of The Total Environment, Volume 814, 2022, 152460

### **3.22.P-Tu332 Generation Rates of Nano- and Microplastics from Four Thermoplastics by Sunlight-simulated Photooxidation in Water**

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The production mechanisms of secondary nanoplastic (NP) and microplastic (MP), and the changes in the surface properties during weathering process have been actively studied recently. However, compared to parent plastics, information on these generated particles remains relatively scarce, particularly regarding particle quantification. This study investigated the changes in surface characteristics of four thermoplastics [polypropylene (PP), high density polyethylene (HDPE), polystyrene (PS) and polyethylene terephthalate (PET)] after photooxidation in water, as well as quantified the generated NPs and MPs and calculated their generation rates. The photooxidation was performed using a xenon-arc lamp with a daylight filter to simulate natural sunlight. The weathering under the same conditions enabled direct comparison between four plastics.

PET showed the earliest signs of surface cracks among the tested polymers, followed by PP and PS. Notably, HDPE did not exhibit any surface cracks even after 176 days of photooxidation. The carbonyl index (CI) of HDPE showed gradually increase with increasing exposure duration. Conversely, the CI of PP, PS, and PET initially increased, but then tends to decrease or fluctuate. Given that the weathering of HDPE is relatively slow compared to other plastics, it is possible that the CI of HDPE will show fluctuation with further weathering progresses. The CI could decrease with exposure time due to weathered surface layers, revealing fresh, unoxidized surfaces underneath. The generation rates ( $y$ ) of NPs and MPs per  $\text{cm}^2$  for  $n$  years after photooxidation and mechanical abrasion (MA) in water were calculated using an exponential function, and each equation is as follows:  $y=30525041 \times e^{0.459 \ln}$  (PP);  $y=8044465 \times e^{0.3939n}$  (HDPE, no MA);  $y=72383709 \times e^{0.5326n}$  (PS);  $y=73005201 \times e^{1.2818n}$  (PET). HDPE for which no equation was derived with photooxidation and MA, was calculated as the case of photooxidation alone.

The NPs and MPs generation rates by photooxidation and MA in this study could provide the basis for developing policies to manage plastic pollution. For example, PET and PS litter is recommended to remove from the environments within 1-1.5 yrs before their exponential increment of microplastic production, and to use HDPE polymer for outdoor plastic facilities to reduce microplastic generation. In addition, they can be used as essential parameters to develop plastic weathering or fragmentation models.

### **3.22.P-Tu333 Environmental Fate of Nano- and Small Microplastics in Aqueous Environments**

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Understanding how micro- and nanoplastics (MP and NP) behave in the environment, and how different environmental conditions impact their fate is fundamental to quantifying exposures, assessing hazards and, ultimately, understanding their risks. Unfortunately, there remains a paucity of data on this topic, particularly factoring in environmentally realistic and relevant particle polymers, shapes, and conditions.

Here, we studied the environmental fate of secondary small MP (sMP; 1-100  $\mu\text{m}$ ) and NP (<1  $\mu\text{m}$ ) in various aquatic matrices. Low-density polyethylene (LDPE) shopping bags and polyethylene terephthalate (PET), polyamide (PA), polyacrylic (PAN) and wool yarns were the primary plastics from which the secondary MP and NP were produced. The sMP (diameter range  $\sim$ 10-80  $\mu\text{m}$ ) were obtained by cryomilling and sieving, while NPs (diameter  $\sim$ 200-500 nm) were obtained directly in water by partial dissolution/precipitation using a long-chain alkane. PE sMP were partially degraded under simulated UV exposure. Particle size distribution (NTA and Coulter Counter), shape and morphology (SEM), and surface chemistry (Raman) was characterized.

Environmental fate experiments were conducted in natural fresh-, brackish and seawater collected from around the Trondheim area (Norway). For NP, the impact of varying environmental factors such as salinity, temperature and organic matter type and concentration on suspension stability and aggregation was studied. Dynamic light scattering (DLS) and zeta potential was measured over a time-series. For sMP, the impact of aging in natural waters with varying content (type and concentration) of



organic matter and microorganisms (phytoplankton and bacteria), as well as UV-degradation, on particle dispersion and settling rates, was studied.

### **3.22.P-Tu334 Are microplastics derived from conventional and biodegradable mulching films biodegradable in the aquatic environment?**

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Mulching films are widely used in agriculture to increase quality and yield of crops. When applied to the soil, they are exposed to various environmental factors that lead to fragmentation and the formation of smaller plastic particles known as microplastics (MPs). The resulting MPs can remain in the soil and affect its quality or be transported by runoff into surface waters, where the fate of MPs derived from agricultural mulching films has not yet been investigated.

In this context, we investigated how the most commonly used mulching films biodegrade in the aquatic environment. For this experiment conventional low-density polyethylene (PE) and biodegradable polybutylene adipate terephthalate (PBAT) mulching films were selected. The untreated MPs (both PE and PBAT) were produced by shredding and cryomilling larger pieces of film. In addition, both untreated MPs were aged (10 days, UVA340 lamps, UV intensity 35-40 W/m<sup>2</sup>) to stimulate the natural abiotic aging under sunlight. The biodegradability in the aquatic environment of all four types of MPs (PE, PBAT, UV-PE, UV-PBAT) was evaluated by measuring the oxygen demand in a closed respirometer over 28 days according to the standardized OECD method. In addition, the changes in morphology and chemical composition after abiotic (UV) and biotic (biodegradability test) aging were investigated using scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR), respectively.

The results showed that PE did not degrade in the aquatic environment, even when pre-treated with UV, as biodegradability was less than 1% after 28 days. In addition, SEM images and FTIR spectra showed no changes in morphology and chemical composition. On the other hand, PBAT was not well degraded (the biodegradability was approximately 6%), while UV-PBAT was partially degraded and reached a biodegradability of 16%. These results are also consistent with the FTIR and SEM analysis, as the FTIR analysis showed changes in the peaks at a wavenumber of 2900 cm<sup>-1</sup> (methylene stretching vibration) and the SEM images revealed disruption of the MPs surface.

The results show that none of the tested MPs can be considered readily biodegradable in the aquatic environment. Nevertheless, PBAT showed an initial degree of degradation in the aquatic environment, but further studies are needed to investigate the mechanisms and time frame of its biodegradation.

### **3.22.P-Tu335 Activated Sludge Acts as an Efficient Passive Sampler for Microplastics**

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Quantifying microplastics (MPs) in wastewater treatment plants (WWTPs) remains challenging due to large uncertainties associated with sampling and analysis. However, high removals of MPs in WWTPs indicate the transfer of MPs from the wastewater to the sludge, suggesting that sludge is an efficient passive sampler for MPs. In this work, we assess the accumulation of pristine and UV-exposed MPs in activated sludge. We performed batch experiments to mimic the behavior of MPs in an activated sludge process. Polystyrene (PS), polyethylene (PE), and polyvinyl chloride (PVC) reference MPs of various sizes, shapes, and densities were exposed to UV light. Approximately 5'000 pristine or UV-exposed MPs were spiked separately in bottles with deionized water (blanks) or activated sludge. The content of the bottles was aerated and mixed (120 min), before settling (30 min) in a sedimentation funnel. The resulting supernatant and settled sludge were digested or filtered and the MPs were quantified based on microscopic images. Surrogate standards were spiked in each sample as QA/QC measure. To assess the kinetics of MPs accumulation in sludge, time-resolved batch experiments were carried out with different aeration and mixing times (0, 1, 5, 15, 30, 60 and 120 min), but with the same settling time (30 min). For all samples, the recoveries of the surrogate standards was >70%, indicating limited MPs losses during the sample processing. In the blanks, after 120 min mixing, <50% of the each polymer type settled at the bottom of the funnel. When adding sludge, >90% of the pristine and UV-exposed MPs were captured in the settled sludge, because of the combined effect of fluid shear and differential sedimentation of the flocs and particles. The time-resolved experiments showed that the MPs got increasingly heteroaggregated with sludge during the first 15 to 30 minutes, before reaching a steady state. The relative affinity of MPs with sludge was similar for every polymer type. In conclusion, MPs quickly accumulated in activated sludge, independently of their properties (size, shape, polymer type, aging). Because of the higher solids concentration, longer retention time and continuous mixing of sludge in full-scale WWTP, collecting sludge samples may be more representative than sampling effluent water. Thus, sludge is a very promising passive sampler for MPs wastewater.

### **3.22.P-Tu336 Long-term Monitoring of Natural Weathering of Microplastics. Study of the Behavior of Adsorbed Metals**

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The presence of plastics in the environment has generated great interest in recent years due to their ubiquity in different environmental compartments. Plastics can be affected by natural environmental conditions, such as ocean currents, solar radiation, temperature variation, salinity or colonization by a wide range of organisms. In general, they will slowly fragment into smaller pieces known as microplastics (MPs) (< 5 mm in size), which will also continue to fragment into even smaller pieces known as nanoplastics (<1 µm in size). MPs have a small size and because of their high hydrophobicity and large surface areas can serve as carrier of pollutants, containing organic contaminants and heavy metals.

As part of the MicroplastiX project's research activities, in-situ experiments were carried out simultaneously in 5 port sites in Europe and in an extra location in Brazil. The experiment aims to assess degradation, fragmentation and weathering of different polymers under environmental conditions.

This study presents the natural weathering study of 9 types of plastics to monitor the changes in the composition of their metallic additives. The weathering experiment has been carried out during the summer, autumn, winter and spring of 2021-2022 in a sport port in Ares, A Coruña, Spain. Subsamples were analyzed at each season at 0, 30, 60 and 90 days of exposure. 15 metals were simultaneously measured by ICP-MS after microwave acid digestion.

As an example, for R-PP, the concentration of some metals such as Mn or Ca increases slightly with exposure time. However, in the case of Cr, it seems to leach. It is the polymer that shows the highest levels of metals at T0. In the case of PLA, there is almost no metal content at T0, while after 90 days of exposure for most of the metals studied, levels above the detection limit are observed. A similar behavior to that of PLA is observed for HDPE. It only presents Ca and Fe at T0 and the metals found increase with exposure time. The levels found in summer are slightly higher than those found in the other three seasons.

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### **3.22.P-Tu337 Microplastics Settling in Turbid Waters: Impacts of Natural Suspended Sediments on Deposition Rates** *Francesco Parrella<sup>1</sup>, Stefano Brizzolara<sup>1</sup>, Markus Holzner<sup>2</sup> and Denise M Mitrano<sup>3</sup>, (1)ETH Zurich, Switzerland, (2)Swiss Federal Research Institute WSL, Switzerland, (3)Environmental Systems Science, ETH Zurich, Switzerland*

Numerous studies have focused on microplastics (MPs) detection and identification in natural waters, but few have investigated their transport behavior (e.g., settling dynamics) in aquatic environments. When MPs enter water bodies, factors such as particle size, density, and interactions with other particles influence their sedimentation rate. Here, we assessed how the presence of small, natural, suspended sediments (clay and silt) at varying concentrations impact MPs deposition in turbid waters. Our goal was to quantify how sediment concentrations alter MPs settling rates compared to pure water and subsequently evaluate deviations from natural particle settling models. We used model irregular-shaped PET fragments in three size classes (25-63 µm, 63-125 µm, 125-250 µm) stained with Nile Red for easier identification. Sediment suspensions at three concentrations (5 mg/L, 15 mg/L, 30 mg/L) were created by mixing kaolin, montmorillonite, and quartz flour in equal proportions. A 1.5 m high plexiglass column, illuminated by a laser and filled with synthetic freshwater and the sediments, was used for settling experiments. A system with four cameras was used to track several thousands of particle trajectories, providing robust data for settling velocities. In line with expectations, larger MPs settled faster than smaller ones at a fixed sediment concentration of 15 mg/L. Surprisingly, MPs settled faster in the presence of suspended sediments compared to pure water, irrespective of sediments concentration. While further investigations are still ongoing, we hypothesize that the perturbation flow generated by falling MPs affected the spatial distribution of clay and silt particles, channeling MPs and speeding up their deposition. In a follow-up experiment, we explored the influence of different sediment concentrations on MPs of a given size. While MPs still settled faster in the presence of suspended sediments, there was no significant impact of different sediment concentrations, possibly due to the limited range of turbidity investigated at this stage of research. Ongoing analyses will explore higher and lower sediment concentrations, as well as other parameters, such as differences in salinity. Once completed, collectively our results will provide valuable data for future MPs fate-models and help to further understand the processes of sedimentation and fate of MPs in natural waters.

### **3.22.P-Tu338 Preliminary field study: vertical difference of microplastics within aggregates from the deep ocean** *Mi Jang<sup>1</sup>, Gi Myung Han<sup>1</sup>, Sung Yong Ha<sup>1</sup>, Youna Cho<sup>1</sup>, Ha Young Cho<sup>2</sup> and Sang Hee Hong<sup>1</sup>, (1)Ecological Risk Research Department, Korea Institute of Ocean Science and Technology, Korea, Republic of (South), (2)KIOST*

In this study, we aimed to characterize marine aggregate and microplastic at the surface and deep sea from East sea (sea of Japan). Thirty litter of seawater was collected from the surface (3m) and deep sea (2100m) by niskin bottle respectively, and samples were obtained by settling seawater for two hours in Imhoff cone. For all samples, aggregates and organisms (mostly planktons) were photographed under microscope, and segregated to separate glass bottles. The seawater (loose fraction) which have aggregates and organisms excluded, and the aggregates and organism fractions were pretreated and placed onto silicon

filter paper for FT-IR analysis of microplastics. The size of aggregates varied from 200 to 5,390  $\mu\text{m}$ , and their concentration was higher at the surface (3900  $\text{n}/\text{m}^3$ ) than at the deep sea (1833  $\text{n}/\text{m}^3$ ). Organisms were only observed in the surface water (5467  $\text{n}/\text{m}^3$ ), and none in deep water. Microplastics were detected in 4 (3%) of 117 aggregates from 30 L of surface water, and 8 (15%) of 55 aggregates of 30 L of deep water. Of the 164 organisms in 30 L of surface water, microplastics were detected in 12 (11%). However, most of the microplastics were in the loose fraction (surface: 96 in 30 L, deep: 36 in 30 L) among the water column. At the surface level, organisms showed a higher proportion of microplastics compared to aggregates. But as we delve deeper, the proportion of microplastic in aggregates increased as the appearances of organisms diminished. This suggested the indirect influence of organisms such as plankton on the settling of microplastics in oceans. The results from this study points out the need for further field monitoring of aggregates in deep oceans.

### **3.22.P-Tu339 The fate of low density microplastic particles in rivers: an experimental study of turbulence, biofilm and sediment**

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In watersheds, microplastic particles (MP) fate in the air-soil-water continuum impacts aquatic environments, ultimately transporting these particles to the seas. The transfer of MP from densely populated and industrialised areas to watercourses is particularly important but, some rivers crossing these types of territories present no significant MP concentration difference between upstream and downstream. In general, three key mechanisms are hypothesized as governing the MP fate in aquatic environments: particle characteristics, interactions with biota, and hydrodynamics. As low-density MP are often found in sediments the role of these mechanisms on the dynamics of this MP dynamics in running water systems is yet to be determined. This study, in an experimental approach, integrates the water column and sediment compartments, exploring the role of turbulence and biofilm on low-density MP dynamics. In a flume containing sediment and biofilm, the trajectory of standard particles of polyethylene polymer (red-fluorescent spheres, 0.995  $\text{g}/\text{cm}^3$ ,  $\sim 50 \mu\text{m}$ ) were followed at different water flow velocities. A Particle Image Velocimeter setup (PIV) followed by data processing using Particle Tracking Velocimetry (PTV) allowed to retrace the trajectory of the particles near the sediment providing their orientations, origins and velocities. Increasing turbulence led to higher relative numbers of MP being in contact with the biofilm from  $\sim 4\%$  to 10%. It enhanced MP settling requiring lower MP concentrations. Red-fluorescent MP were spotted on the biofilm surface but also in flocs suspended in the water column. In total, 6.000 particles  $\text{cm}^{-2}$  were observed in flocs, while 4.000 particles  $\text{cm}^{-2}$  were present in the water. In the complex interaction of the different parameters driving MP in the watercolumn of rivers and streams, the turbulence and the biofilm significantly affect the settling of low-density MP not only on the sediment but also in the water column by biofilm flocs. Further comprehension of biofilm-turbulence role on MP dynamics increase understanding on spatio-temporal variation of MP settling, transport and, thus, fluxes.

### **3.22.P-Tu340 Metal-Organic Frameworks (MOFs) as an Effective Tool for Nanoplastic Removal from Water**

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Nanoplastics (NPs) have attracted significant global attention as emerging environmental pollutants, given their heightened susceptibility to organismal internalization, raising concerns about increased ecological and health risks compared to microplastics (MPs). Recently, the focus has shifted towards adsorption as a promising strategy for NP removal, with new adsorbents demonstrating impressive performance. In this study, we aimed to eliminate polystyrene nanoplastics from aqueous environments using a series of mesoporous Metal-Organic Frameworks (MOFs). Mesoporous UiO-66 and its derivatives (-OH and -NH<sub>2</sub>) were synthesized through a direct solvothermal process, employing cetyltrimethylammonium bromide (CTAB) or Pluronic-type triblock copolymer (P123). The resulting materials exhibited high crystallinity and featured a novel hierarchical mesoporosity.

TEM images provided evidence that NPs did not enter the mesopores of the material; instead, they predominantly adhered to the external surface of the MOF. Despite this, the mesoporosity of the MOF played a crucial role in augmenting the active surface area, positively influencing the adsorption process. This finding underscores the importance of mesoporosity in enhancing the overall adsorptive capacity of the MOF, even when NPs primarily interact with the external surface rather than entering the mesopores. Particularly noteworthy was the exceptional efficiency of UiO-66-NH<sub>2</sub>/P123 in NP removal, achieving up to 100% removal efficiency at an initial concentration of 1  $\text{g}\cdot\text{L}^{-1}$ . This outcome underscores the potential of MOFs as highly effective adsorbents for removing NPs from aqueous media.

### **3.22.P-Tu341 Predicting the Toxicity of Untested Microplastic Particles**

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Micro- and nanoplastic particles (MNPs) are highly complex. While chemicals can be assigned unique identifiers (e.g. CAS numbers, SMILES) based on their clearly defined molecular structure and stable properties, this is not possible for MNPs. Each MNP has its own set of traits, including characteristics such as polymer composition, particle size and shape, as well as physicochemical properties like surface charge, surface chemistry, and plastic-associated chemicals. Furthermore, all these traits can change over time, for instance through degradation processes when MNPs are exposed to natural environments. To achieve reliable hazard and risk assessments for MNPs, it may be necessary to predict the toxicity of MNPs with trait combinations that have not been tested directly in the lab. Like Quantitative Structure-Activity Relationship (QSAR) models that link molecular structures to toxic outcomes, models are needed to link MNPs traits to toxicity.

The recently compiled Toxicity of Microplastics Explorer (ToMEx) 2.0 database, consisting of 12,774 data points from 215 published studies of MNP effects on aquatic species, offers a unique opportunity to approach this task. Using ToMEx 2.0 data, we train machine learning models on tasks to predict the toxicity (presence/absence of effects, effect direction, effective concentrations) of untested MNPs via leave-one-out cross validation. We compare the predictive performance of different machine learning algorithms and use methods of explainable AI (average marginal effects) to gain insights into associations of toxic outcomes with MNPs traits, experimental parameters, and species traits. Finally, we discuss how such models can be used to predict the toxicity of environmentally relevant MNPs mixtures and to inform the development of less toxic and more environmentally friendly plastic materials in the future.

### **3.22.P-Tu342 OCCURRENCE OF COMMON PLASTIC ADDITIVES AND CONTAMINANTS IN RAW, STEAMED AND CANNED MUSSEL SAMPLES FROM DIFFERENT HARVESTING AREAS USING MSPD-HPLC METHODOLOGY**

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Microplastics are a complex mix of chemicals containing polymers and certain plastic additives such as bisphenols and phthalates. These particles are porous materials that can also sorb contaminants from their surroundings, and leach chemicals from the particle under certain circumstances. Aquatic animals can ingest microplastic particles, which mostly bioaccumulate in the gastrointestinal tract of animals. In terms of dietary exposure, small animals consumed whole such as mussels, contribute more to the dietary intake of microplastic particles. Plastic additives and contaminants are not chemically bound to the polymers, and certain processing methods or cooking processes result in the release of these chemicals that leach from the plastic particles, leaving them more available for absorption when ingested.

In this work, the effect of cooking and processing in the presence of compounds derived from microplastic contamination in mussels was studied. For this purpose, an analytical methodology based on matrix solid phase dispersion (MSPD) coupled with high-performance liquid chromatography-mass spectrometry (HPLC-MS) was developed for the simultaneous determination of nine analytes (di (2-ethylhexil) phthalate (DEHP), dibutyl phthalate (DBP), diethyl phthalate (DEP), bisphenol A (BPA), bisphenol F (BPF), bisphenol S (BPS), dichlorodiphenyldichloroethane (DDD), dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyldichloroethylene (DDE)] in raw, steamed, and canned mussels samples of two different harvesting areas (Atlantic and the Mediterranean).

MSPD sample treatments provided satisfactory recoveries for all the analytes and for raw, steamed, and canned mussel samples coming from the two studied areas, being the recoveries between 81–90% (with Relative Standard Deviation, RSD, of 1.7-8.4%) for steamed samples, and between 77-84% (RSD between 1.3-19%) for canned samples, and the detection and the quantification limits between 0.06-4.63 µg/kg. The results showed that the heat and pressure treatment could influence the migration of plastic additives as bisphenols and phthalates from the microplastic particles present in mussels to the processing liquid (water and/or oil), having as a result the decrease in the concentration of analytes in the food matrix. Analytes were always found to be higher in raw samples, followed by steamed. Only DBP and DEP were detected in canned samples. The pesticides DDD, DDT, and DDE were not detected in any of the cases.

### **3.22.P-Tu343 Leaching of Dissolved Organics and Inorganics From Microplastics and Assessing Their Contribution to Disinfection Byproducts Formation**

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The widespread use of plastics in daily lives has increased the occurrence of microplastics (MPs) in water and wastewater. While much research has been conducted on MP occurrence, additive leaching from MPs, and their adverse effects, relatively little is known about the potential leaching of dissolved organic matter (DOM) from MPs. This study aims to fill that research gap by investigating DOM and inorganics leaching from MPs, including polyamide 6, high-density polyethylene, and polyurethane under accelerated UVC irradiation. Leaching from these MPs was analyzed using gas chromatography-mass spectroscopy and elemental analysis. Finally, their potential role in the formation of disinfection byproducts (DBPs) was evaluated. Under ten days of UVC irradiation, MPs exhibited high levels of dissolved organic carbon and nitrogen leaching, reaching up to 94.1 and 29 mg/L, respectively. The leachates contained various metals, including Na, Mg, Al, K, Ca, Fe, and Cu. Metalloids like Si, and reactive nonmetals, such as P, Cl, and Br, were also detected. Among them, Na, P, K, Cl, and Si

were the most frequently detected, with Na reaching the highest levels up to approximately 60.5 mg/g-MP. This suggests that MPs can be a notable contributor to the influx of not only organic compounds but also metals into natural water bodies. Chloramination of leachate did not yield detectable levels of *N*-nitrosodimethylamine or any regulated DBPs. Nonetheless, the chloramination process produced butanedinitrile and diacetamide, which are considered potentially toxic non-halogenated DBPs. Furthermore, chlorination of leachate revealed the formation of unregulated DBPs, such as chloral hydrate and propane nitrile. Given the formation of potentially toxic DBPs during the disinfection of DOM leached from MPs, it is recommended to include the consideration of DOM leaching from MPs as DBP precursors in toxicity analyses. This includes evaluating the toxicity of both the leached DOM and the resulting DBPs. Our results indicate that the leaching of DOM from MPs in aquatic environments may contribute to the formation of non-regulated DBPs during the disinfection process. These findings highlight the importance of monitoring DBP formation from MPs to safeguard both drinking water and the aquatic environment. Overall, the research enhances our understanding of the overlooked negative impacts associated with microplastic pollution in aquatic environments.

### **3.22.P-Tu344 Leaching of plastic additives in seawater: role of biofilm in colonized plastic**

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In seawater, plastic leaches out chemical compounds which has implications for the marine fauna and the biogeochemistry of the seawater. These chemicals can be additives or degradation products of the polymer and the process is enhanced by sunlight radiation. The presence of microorganisms in seawater also triggers a fast formation of a complex biological layer on the plastic surface known as 'biofilm'. This is composed of a blend of microorganisms, extracellular compounds, and organic matter adhering to the plastic surface, fundamentally altering its properties and interactions within the environment. All the studies analyzing plastic leaching process in seawater have been made using new virgin plastic ignoring the role of the biofilm in this process. This study explores this phenomenon to understand if the presence of biofilm on plastic enhances or inhibits the leaching process in seawater.

Two experiments were performed to study the plastic leaching of additives in seawater: one with low density polyethylene (LDPE) colonized by biofilm and other, as a control, with new LDPE without biofilm. Plastic pieces were incubated in sterilized seawater under artificial UV and Vis radiation. In the experiment with non-colonized plastic, dark treatments were also performed to study the effect of radiation on the leaching. Samples from water, plastic, and biofilm were meticulously collected at specific intervals. Organophosphates (OPEs) and phthalates (PAEs) released by plastic into seawater were analyzed using Agilent 7890A gas chromatograph coupled to an Agilent 7000B triple quadrupole mass spectrometer.

The experiment with non-colonized plastic revealed substantial concentrations of organophosphates (OPEs) and phthalates (PAEs), indicating their potential for leaching under diverse light and darkness conditions. A notable observation was the distinct response among various OPEs to UV exposure, contrasting with the consistent behavior observed in PAEs. These results will be compared with those obtained from the experiment with colonized plastic. Findings from this study are expected to offer critical perspectives in comprehending the complex dynamics of chemical leaching associated with plastics in marine environments.

### **3.22.P-Tu345 Microplastics interaction on Hg biotransformations mediated by microorganisms**

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The presence of microplastics ( $\emptyset < 5$  mm, MPs) and mercury (Hg) in aquatic ecosystems is an environmental problem. MPs can be taken up by biota, undergo bioaccumulation and biomagnification and cause oxidative stress, neurotoxicity, genotoxicity, and inflammation. Microorganisms are the key players in the biogeochemical cycle of Hg, performing Hg-reduction, Hg-methylation (formation of the neurotoxic methylmercury), and methylmercury-demethylation. Methylmercury undergo bioaccumulation and biomagnification. Microorganisms isolated from Tagus estuary, one of the most important wetlands of Europe, were found to be involved in these processes. Thus, this study aims to assess the complex interactions between MPs, Hg and Hg-resistant microorganisms to disclose the impacts of MPs on the biogeochemical cycle of Hg and toxicity.

To accomplish this, an urban area of Tagus estuary with port and industrial activities, was selected as study case (Barreiro, 38.6645,-9.0786). Sediment and water samples were sampled for: (1) Microplastics isolation and characterization; (2) total Hg concentration determination, and (3) Hg-resistant microbial communities' isolation. Besides, DNA was extracted from the MPs and sediment samples to assess the presence of Bacteria, Fungi and Archaea via PCR for the amplification of 16S rRNA and

ITS genes. Suspension and microcosms experiments with several MPs polymers with  $\text{Ø} > 1$  mm were performed to evaluate the interactions between MPs:Hg:Microorganisms.

The results showed the presence of MPs ( $109 \pm 71.6$  to  $52.0 \pm 8.70$  particles/kg of dry sediment), especially fibers and fragments of polypropylene, polyethylene, expanded polystyrene and polyester polymers. Hg concentration above Effects Range Medium (0.71

### **3.22.P-Tu346 Joint effects of ketoprofen and polypropylene nanoplastics on the morphology and physiology of zebrafish early life stages**

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Ketoprofen (Kp) is a non-steroidal anti-inflammatory medicine commonly used worldwide; however, there is no clear legislation regarding its emission into the environment. Concentrations in environmental matrices, particularly in water resources, are known; although their effects on the biota have received less attention. They are likely to affect the health and performance of fish because of their powerful and enduring analgesic and anti-inflammatory effects. Furthermore, in realistic environmental contexts, this drug is possible to interact with emerging compounds, such as nanoplastics (NPLs), which may reduce or potentiate its uptake and, consequently, influence the observed toxicity. Accordingly, two specific objectives were pursued in the present study: I) to quantify the lethal concentrations of Kp causing 20 and 50% of effect (LC<sub>20</sub> and LC<sub>50</sub>, respectively) on embryos and larvae of *D. rerio* and II) to understand how the toxicity of Kp (at two levels: LC<sub>20</sub>, LC<sub>50</sub>) is shaped by the presence of environmentally realistic concentrations of nanosized polypropylene plastic (PP, 25-101 nm at 0.0001, 0.001, 0.01 mg/L), on a full factorial design. The following endpoints were monitored: mortality (M), malformations (m), heart beats (Hb), hatching rate (Hr), total length (TL) and interocular distance (IO). The PP was selected because it is widely used for food packaging and is one of the most often found in rivers. The estimated LC<sub>20</sub> and LC<sub>50</sub> (and respective confidence limits at 95%) for larvae were of 3.52 (2.31 - 5.35) mg Kp/L and 10.09 (6.63 - 15.35) mg Kp/L, respectively, and for embryo were of 4.29 (3.10 - 5.12) mg Kp/L and 6.16 (5.17 - 7.43) mg Kp/L. In plastics' exposure alone, M+m were below 20% in both life stages. For larvae, the LC<sub>20,Kp</sub> and LC<sub>50,Kp</sub>, M+m summed up to 20 and 35% of effect, whilst up to 30% in LC<sub>20,Kp</sub>+0.01 mg PP-NPLs/L, and up to 40% in LC<sub>50,Kp</sub>+all PP-NPLs concentrations. The M+m for embryos, jointly summed up to 35% and 50% of effect in LC<sub>20,Kp</sub> and LC<sub>50,Kp</sub> groups. Significant differences were observed for IO between control treatments (except 0.001; 0.01 mg PP-NPLs/L and LC<sub>50,Kp</sub>+0.01mg PP-NPLs/L) and also for Hb, significant differences were observed between LC<sub>50,Kp</sub> and control. These results highlight that some potential synergistic interaction between pharmaceuticals and NPLs may occur, hindering the true effects of NPLs when studying them isolated.

### **3.22.P-Tu347 Effect of low food level on chronic toxicity of polyethylene microplastics to Daphnia magna population** Zhihan Cao, Changhae Kim and Jinho Jung, Environmental Science & Ecological Engineering, Korea University, Korea, Republic of (South)

Food level has a vital role in population dynamics. However, the role of food level in *D. magna* population dynamics with microplastics (MPs) is not fully understood. In this study, populations of *D. magna* were exposed to polyethylene MP fragments, BP-3 leachate, and MP fragments containing the UV stabilizer benzophenone-3 (MP/BP-3). The food concentrations were  $5 \times 10^5$  cells/mL (optimum level) and  $2 \times 10^5$  cells/mL (low level). The test duration was 42 days to observe both the growth and decline of *D. magna* population. Low food levels itself significantly decreased the population size and changed the population structure ( $p < 0.05$ ). Under the low food levels, both MP and MP/BP-3 fragments significantly decreased the population size in the decline phase ( $p < 0.05$ ), but there was no difference between them. Interestingly, BP-3 leachate delayed the *D. magna* development, causing a significant decrease in the population size from the growth phase ( $p < 0.05$ ). These findings suggest that BP-3 contained in MP/BP-3 fragments was not likely available to *D. magna*. However, the interaction mechanism between MP and BP-3 needs to be further studied.

### **3.22.P-Tu348 Toxicity of Micro- and Nanoplastic Particles on Daphnia: A Meta-Analysis of the Effects of Dissolved Organic Matter and Ecocorona Formation**

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The toxicity of micro- and nanoplastics (MNPs) has gained attention in recent years, with water fleas of the *Daphnia* being the most tested aquatic invertebrate. However, the majority of studies have focused on the impact of pristine MNPs, neglecting the ecocorona that forms when organic molecules attach to MNP particle surfaces in natural environments. It remains unclear whether ecocorona formation plays a role in mitigating the toxic outcomes of MNP on aquatic species. To address this gap, we conducted a meta-analysis, specifically examining the immobilization rates of *Daphnia* exposed to MNP particles in the presence of dissolved organic matter (DOM) and ecocorona formation. A systematic literature search identified relevant experimental studies, allowing us to compare the effects of MNPs with ecocorona/DOM to those without. Our analysis considered variations in DOM types and experimental approaches (either co-exposure with DOM or incubation of MNP prior to exposure).

The final search was conducted in December 2022 on Web of Science (WoS) and PubMed. In total, 305 data points from 13 publications were included, revealing that DOM has the potential to alleviate the adverse effects of MNPs on *Daphnia* immobilization. Contrary to some hypotheses suggesting the primary role of ecocorona alone, our findings suggest that DOM in the media contributes additionally to the observed mitigation. Furthermore, the moderating effects of DOM varied based on the type of DOM utilized. Humic acid and lake water had the strongest mitigating effects, reducing the risk of immobilization by nearly 50%.

Based on our findings and literature on other stressors, we hypothesize that DOM mitigates MNP effects by either (1) reducing MNP bioavailability or (2) enhancing the daphnids' general resilience to stressors. Future studies comparing the impact of ecocorona formation during MNP incubation with adding the same type of DOM to the media during exposure will help to untangle the moderating effects of the ecocorona itself and free DOM in the media. In addition, studies comparing different DOM types or investigating interactions with additional stressors, and the more detailed characterization of MNP availability to *Daphnia* in the presence of DOM will improve our mechanistic understanding in the future.

### **3.22.P-Tu349 The Clone Wars: *Daphnia magna* Clones React Differently To Microplastics Exposure Under Food Limitation**

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Ecotoxicological investigations of microplastic (MP) often report a wide variety of effects, even when relying on a model organism, like *Daphnia magna*, that reproduces via amictic parthenogenesis and should so enable researchers to exclude genetic variability. This could certainly be attributed in part to the diverse physicochemical properties of different MP, however MP ecotoxicological studies on *D. magna* often fail to consider clonal genetic variability, as well as other interacting stressors. In fact, in the environment, parameters like e.g. temperature, light, food availability are not constant. Food levels in particular are often far from those usually provided in laboratory investigations, hence we decided to perform this experiment in a food limiting set up for more environmental relevance (0.5 mg C L<sup>-1</sup> every second day). The aim of this work is therefore to compare the sensitivity of two *D. magna* clonal strains (Aig and BL2.2) when exposed to MP under food limitation. For the MP exposure we chose to compare the potential effects induced by fragments of two biodegradable polymers (PLA and PBS) to those of a petroleum-based one (PET). Microcrystalline cellulose fragments were included as reference particles (all fragments < 20 µm). We carried out a 21-day chronic exposure experiment during which we recorded life history traits, growth and mortality. The two selected *D. magna* clones were found to have significantly different responses in all the selected sublethal parameters, even when statistically excluding intrinsic clonal differences by centering the obtained results to each separate clonal control, with Aig clone being more sensitive than BL2.2. Furthermore, when considering the effects of petroleum-based MP compared to those induced by biodegradable ones, the latter induced comparable or even higher negative effects. Therefore we conclude that clonal variability can significantly affect exposure studies and this should be considered in MP research. Moreover, despite evidence already exists that biodegradable polymers can have similarly negative effects as commodity plastics, further assessments are still needed to support these findings.

### **3.22.P-Tu351 Combined effects of global warming and microplastic on the population dynamics of a harpacticoid copepod**

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Global warming and plastic pollution are two human-induced environmental stressors of concern due to their potential impact on ocean health. Microplastic is ubiquitously and persistently present in the marine environment, yet knowledge of its effects on populations, especially in a warmed marine environment, is limited. Here, our goal was to assess the potential theoretical population effects of microplastic and global warming. To do so, we followed a two-step approach: First, we exposed the harpacticoid copepod *Nitokra spinipes*, a benthic copepod, to Poly (lactic-co-glycolic acid) (PLGA) microbeads (5 µm), at control (22°C) and increased (25°C) water temperatures. The effects on *N. spinipes* individual were assessed by identifying shifts on filtration rate on microalgal prey, a proxy for energy assimilation. In the next step, the dynamics of a *N. spinipes* population was simulated for projected global warming conditions, and the effects of microplastics on the population density equilibrium were assessed. The empirical filtration rate data was incorporated in an individual-based model implementation of the dynamic energy budget theory (DEB-IBM model) to deduct potential theoretical population level effects (ongoing analysis). Our preliminary results suggest that PLGA microbeads at 0.1 % food content decreased the filtration rate of *N. spinipes* at increased water temperature (25°C) ( $P < 0.05$ , ANOVA). All *N. spinipes* at the increased water temperature (25°C) have a higher filtration rate compared to the control temperature (22°C). Our study demonstrates that the combined exposure of microplastic and elevated water temperature can induce less energy assimilation, in a high emissions scenario (RCP 8.5, IPCC) and inform the vulnerability of marine populations under current and future environmental conditions.

### 3.22.P-Tu352 Release of Microplastics from Disposable Surgical Facemasks Under Varying Shear Forces and Its Toxic Effects On Freshwater Algae *Scenedesmus Obliquus*

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Throughout the COVID-19 pandemic, an extensive quantity of surgical face masks was used, as they emerged as an essential need in the daily routines of individuals worldwide. However, these items were not appropriately discarded, constituting a major contributor to micro and nano plastics throughout the environment. The impact of mask-derived microplastics on aquatic organisms has yet to be extensively investigated. This study aimed to quantify and describe the microplastics emitted from disposable surgical face masks under varying shear force (100, 200, and 400 rpm). We investigated the acute toxic effects of the released microplastics on freshwater algae *Scenedesmus obliquus*. The findings indicated that greater concentrations of microplastics were leached away as shear forces increased from 100 to 400 rpm. Ecotoxicity results showed that a concentration-dependent decrease in cell viability, photosynthetic yield, and electron transport rate was observed in the algal species. This was accompanied by increased oxidative stress markers such as reactive oxygen species (ROS) and lipid peroxidation (LPO). There was also a significant rise in antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in the algal cells. Furthermore, morphological changes like cell aggregation and cell deformity were ascertained by Scanning electron microscopy. Our findings show that surgical face masks emitting microplastics represent a significant environmental threat. Additional research involving various freshwater species is necessary to evaluate the ecological consequences of the leachates from masks.

### 3.22.P-Tu353 Transcriptomic Alteration of *Mytilus galloprovincialis* Exposed to Virgin and Marine Incubated Microparticles Made of Biodegradable and Conventional Polymers

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Biopolymers have been proposed as an alternative to conventional plastics to mitigate the impact of plastic litter, but their use in marine environments has raised concerns, especially regarding the potential toxicity to marine life. This study evaluates the potential ecotoxicological effects of both virgin and marine-incubated microparticles (MPs), at relevant environmental concentration (0.1 mg/l), made by different biodegradable polymers (Polycaprolactone, Mater-Bi, cellulose) and conventional polymers (Polyethylene) on *Mytilus galloprovincialis* by using a transcriptomic approach. Whole hepatopancreas *de novo* transcriptome sequencing was performed, individuating 972 genes differentially expressed across experimental groups compared to the control. Overall, through the comparative transcriptomic profiling, emerges that the preponderant effect is attributable to the marine incubation of MPs, especially for incubated polycaprolactone MPs (731 DEGs). These findings highlight the necessity of considering the interactions of MPs with the environmental factors in the marine ecosystem, rather than restrict the ecotoxicological evaluations to virgin material only. All microparticles, regardless of their polymeric composition, dysregulated innate immunity, and fatty acid metabolism biological processes. The results obtained contribute to fill current knowledge gaps regarding the potential environmental impacts of biopolymers and represent a first indication that some bio-based biodegradable polymers may constitute a promising alternative to conventional plastics.

### 3.22.P-Tu354 Mechanistic Assessment of Cellular Responses to Micro- and Nanoplastic Particles (Aged Polyethylene Terephthalate) in Bivalve Hemocytes (*Mytilus edulis*)

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Invertebrate immune cells, particularly hemocytes of bivalve mollusks, play a crucial role in cell-mediated immunity and are valuable models for investigating particle-induced responses. Different subpopulations of hemocytes, such as hyalinocytes and granulocytes, exhibit distinct morphological and functional characteristics, including internal cell signaling pathways that regulate various cellular processes. While there is a growing body of research documenting various biological responses to plastic particle exposure, systematic analyses of effect endpoints at the cellular level remain limited. Hence, this research focuses on assessing the mechanistic interactions of micro- and nanoplastic particles (MPs/NPs) within cellular environments and their influence on the functionality of bivalve hemocytes (*Mytilus edulis*). Two different size ranges of aged polyethylene terephthalate (APET) micro- and nanoplastic particles were utilized: APET MPs ( $D_{50} = 1.4 \mu\text{m}$  and  $D_{90} = 3.1 \mu\text{m}$ ) and APET NPs ( $D_{50} = 0.57 \mu\text{m}$  and  $D_{90} = 1.1 \mu\text{m}$ ). In-vitro experiments were carried out using environmentally relevant concentrations, including low concentration (10 particles/mL), mid concentration ( $10^3$  particles/mL), and high concentration ( $10^5$  particles/mL). A Single Particle Extinction and Scattering (SPES) technique was employed to determine the size distribution and concentration of the particle stock suspension. Prior to the exposure experiments, a secondary characterization of the aged PET was conducted to ensure the stability of the particles under conditions adopted for the in-vitro exposure experiments using Dynamic Light Scattering (DLS). To evaluate the underlying mechanisms induced by plastic exposure at the cellular level, an in-depth assessment was carried out for a series of functional assays using flow cytometry, including cellular viability/mortality, oxidative activity, and lysosome stability. The responses of the hemocyte subpopulations, specifically granulocytes and hyalinocytes were assessed at various time points in-vitro (6 hr, 12 hr, 24 hr, and 48 hr). Differential responses between immune cell types were observed, in particular, lysosome destabilization was evident in the group exposed to the nano-size range plastic particles. The experimental results offer insights into the dose-response relationships for plastic particles at the cellular level.



### 3.22.P-Tu355 Toxicity of a Multi-Polymer Microplastic Mixture on *Mytilus edulis*

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Microplastic (MP) pollution in marine environments is a topic of emerging concern. Due to their ubiquity and small size, plastic particles are easily taken up by marine organisms, causing adverse biological responses. Research so far has focused primarily on the impacts on organisms by pristine test materials that are often spherical and single polymer made. However, plastics found in the environment are mainly a mixture of different polymers with varying sizes and shapes, which raises questions about their combined impacts in marine organisms. Mussels *Mytilus sp.* are widely acknowledged bioindicators in ecotoxicological and biomonitoring studies for evaluating the health status of aquatic ecosystems, mostly due to their wide distribution, filter-feeding ability, sessile behavior, and ecological and commercial importance. Accordingly, the main objective of this study was to investigate the accumulation and toxicity of a MP mixture of relevant polymers (polypropylene, polyamide and polyethylene, <200 µm) at three concentrations (0.1 mg/L, 1 mg/L and 10 mg/L) in the mussel *Mytilus edulis* over a three-week exposure experiment (14 days exposure and 7 days depuration). A battery of cellular, biochemical and physiological indices of stress (biomarkers) was subsequently determined at different time points, including micronuclei formation, DNA damage, acetylcholinesterase activity, antioxidant enzymes activity, lipid peroxidation, cellular energy allocation, histochemistry and digestive enzymes. The occurrence of the MP mix in mussel tissues was also quantified. Analyses are ongoing, but it is envisioned that the results obtained will contribute to understanding the interactions between different polymers in the environment and their potential toxic effects in organisms. Overall, this study will help assessing the toxicity thresholds, as well as establishing baseline hazard knowledge for a MP mixture of environmentally relevant polymers and support future hazard and risk assessment of MPs in Norwegian ecosystems. This work was supported by the MicroOPT Project funded by the Norwegian Retailers Association – Handelens Miljøfond.

### 3.22.P-Tu356 Occurrence and metabolic effects of micro - nanoplastics (MNP) in blue mussels from Isle of Cumbrae, Scotland

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Microplastics (MP) are prevalent in the marine environment. Microplastics (MP) are defined as synthetic polymers less than 5 mm. They have different shapes and forms, such as fibres, spheres, fragments or films and can breakdown into smaller particles known as nanoplastics (NP). MNP have been detected in many marine species. Bivalves are often used to study MNP pollution in the sea as they uptake MNPs from the environment during filter feeding. However, data regarding the accumulation of MNPs in wild bivalves and their impact on whole organismal physiology are still limited. To better understand the metabolic impacts of various types and forms of MNPs, this study measured the oxygen consumption (MO<sub>2</sub>) of depurated wild blue mussels (*Mytilus edulis*) from the Island of Cumbrae, Scotland, a hotspot for MP pollution, in the presence and absence of controlled 7-day MNP exposure. Mussels were exposed to 100nm polystyrene (PS) beads at a low ( $3.64 \times 10^6$  L<sup>-1</sup>) and high concentrations ( $3.64 \times 10^{13}$  L<sup>-1</sup>); to 1 µm PS beads at  $4.55 \times 10^6$  L<sup>-1</sup> and to polyester (PES) fibres ( $1.1 \times 10^4$  mL<sup>-1</sup>). Oxygen consumption was measured using stop-flow respirometry and standard metabolic rate, maximum metabolic rate, and aerobic scope were assessed before and after MNP exposure. We found no impact of MNP exposure metabolic rate under any condition (Before exposure Aerobic Scope; Mean (± SEM)  $35.2 \pm 3.2$ , n = 68) and after exposure  $37.3 \pm 3.2$ , n = 68) (mg O<sub>2</sub> Kg<sup>-1</sup>hr<sup>-1</sup>) (two-way repeated measures ANOVA, p = 0.3117). A second aim was to assess MP accumulation in the tissues of mussel from both the controlled exposure that which occurred in the wild and was not eliminated via depuration. We found environmental microplastics in all organs with an average (± s.d) of  $16.2 \pm 31.5$  (n=88) env-MP g<sup>-1</sup> of tissues with fibres being the majority  $19.4 \pm 34.9$  (n=88) env-MP g<sup>-1</sup>. Mean (± s.d) tissue specific levels were not significantly different (one-way ANOVA, p = 0.088); digestive gland ( $10.3 \pm 11.5$ , n=23), mantle ( $7.6 \pm 8.8$ , n = 22), gonad ( $28.8 \pm 38.7$ , n=25) and gill ( $16.4 \pm 17.6$ , n=26). We conclude that 7-day exposure to MNP's does not impact the metabolism in this species. The ubiquitous presence of MNP from the environment in the tissue prior to collection from the wild may negate any impact of additional MNP burden on metabolism.

### 3.22.P-Tu357 Assessing the toxicity of a multi-polymer and multi-size microplastic mixture on early life stages of Atlantic cod (*Gadus morhua*)

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Despite the research attention that the topic of microplastic (MP) toxicity has received in recent years, knowledge remains limited on the impacts of MPs on organisms at higher levels of biological organisation. To date, research has mainly focused on the effects caused by single polymer materials and by spherical particles with a definite size. However, MPs found in the environment are comprised of a complex mixture of different polymers, with different densities and a continuum of particles shapes and sizes, which raises concerns about their cumulative impacts in marine organisms. From an economic and ecological

perspective, Atlantic cod (*Gadus morhua*) is a key benthopelagic species for many countries, including Norway. Assessing the impacts of MP exposure to *G. morhua*, particularly on the most sensitive and vulnerable early life stages, is critical for understanding risks related to species and ecosystem health. In this study, we investigate the toxicity of irregular-shaped MP with a broad size distribution (1-200  $\mu\text{m}$ ) representing different polymers (PA, PE and PP) on early life stages of *G. morhua*. The different MPs are tested either as individual polymer types or simultaneously as a mixture of different polymers. Each treatment will be tested at three relatively low concentrations 0.1, 1, 10  $\text{mg L}^{-1}$  to ensure a more environmentally relevant MP exposure. Differences in the size, shape and chemical composition of particles were verified using different techniques including G3 morphology analysis, two-dimensional gas chromatography mass spectrometry (GCxGC-MS) and pyrolysis GC-MS. A battery of different toxicological endpoints, including survival, hatching success, developmental alterations, will be assessed, as well as other relevant biomarkers such as oxidative stress and DNA damage. The outcomes of this work are expected to help in establishing toxicity thresholds for MP mixtures, in deconvoluting the single-polymer vs mixture effects, and in establishing baseline knowledge for supporting the environmental risk assessment of MPs in Norwegian marine ecosystems.

### **3.22.P-Tu359 ABUNDANCE AND POTENTIAL TOXICOLOGICAL EFFECTS ON PLANTS OF MICROPLASTICS FROM EL GORGUEL BEACH (MURCIA)**

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One of the environmental problems causing the most concern in recent times is contamination by microplastics, which have been detected in soils, bodies of water (seas, rivers, lakes, and oceans), air, and even in living beings. Determining the level of microplastic contamination in different environmental compartments helps to understand the possible toxic effects that microplastics can have on different organisms.

The beach of El Gorguel (Murcia) is bounded by the Sierra Minera de La Unión and the Sierra Fausilla. This environment is of great ecological importance as a habitat for several species of flora and fauna (e.g. *Sylvia undata*, *Bucanetes githagineus*, *Aquila fasciata*, *Erodium paularense*, *Cheilolophus massonianus*, *Tetraclinis articulata*) catalogued with different degrees of vulnerability according to the IUCN Red List, as well as being home to endemic species (e.g. *Teucrium carthagenensis*, *Limonium carthagenensis*).

For this reason, it belongs to the Natura 2000 Network and has been declared a Site of Community Importance (SCI) and a Special Protection Area for Birds (SPA). However, mining activity has caused this area to be heavily contaminated by heavy metals, but it is also affected by emerging pollutants, such as plastics. Due to the possible interactions that can occur between these pollutants and how they can damage biodiversity, the aim of this work was to determine the presence of plastics on the beach of El Gorguel, as well as their possible toxic effects on the model species: *Solanum lycopersicum* and *Allium cepa*. To this end, the effects caused by exposure to microplastics from plastic plugs found on the beach, as well as leachates from these, on the germination and growth of *Solanum lycopersicum* and *Allium cepa* were evaluated.

The concentrations of microplastics and their leachates used in the tests were selected from the microplastic abundance data found on the beach under study. After 14 days of exposure, the results were analysed. In the case of germination, no significant differences were observed between the control and the different concentrations of the contaminants in either of the two species studied. While elongation of both species was altered by the presence of microplastics, significant differences were observed between the control and some of the higher concentrations of the contaminant. *Allium cepa* was more sensitive to microplastic exposure than *Solanum lycopersicon*.

### **3.22.P-Tu360 Polystyrene and Polyethylene Terephthalate Microplastics Alter Bioaccumulation and Toxicity of Cadmium in the Polychaete, *Perinereis aibuhitensis***

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Heavy metal (HM) pollution in the marine environment has been of concern for decades. The potential impact of HMs carried by emerging marine pollutants such as microplastics (MPs) has attracted attention only in recent years. Sediment is potentially one of the biggest pools of HMs and MPs after their interaction and sedimentation. Nevertheless, the combined effects of HMs and MPs to the benthic infauna in marine environment has received little attention yet. In this study, we select sediment-dwelling ragworm, *Perinereis aibuhitensis*, as model organism to investigate (1) the influences of two types of MPs (polystyrene, PS and polyethylene terephthalate, PET) on the cadmium (Cd) bioaccumulation in worms; (2) the single and combined chronic toxic effects of Cd with PS microspheres or PET microfibers on worms. Our results showed that Cd bioaccumulation was significantly higher with the coexistence of PS than those of Cd alone at the medium ( $\text{Cd}_M$ ) and high ( $\text{Cd}_H$ ) exposure groups. Subcellular fractionation result showed that for the low Cd exposure group ( $\text{Cd}_L$ ), the presence of PET microfibers resulted in Cd proportion in subcellular fractions decreasing from 91.0% to 71.5% in the biologically detoxified metal (BDM) fraction, and increasing from 8.6% to 20.0% and from 8.7% to 20.4% in the metal-sensitive fractions (MSF) fraction and trophically available metal (TAM) fraction, respectively. This indicates that the coexistence of PET contributes to the increase of cell damage and the decrease of cellular detoxification capacity, as well as the increasing bioavailability of Cd through trophic level. In addition, the alteration of Cd proportion in different subcellular fractions by the PET is Cd concentration-related, as the reverse phenomenon was observed in the  $\text{Cd}_M$  group with the presence of PET. Chronic toxicity results demonstrated that  $\text{Cd}_L$  alone significantly decreased the burrowing time of *P. aibuhitensis* in sediment. The presence of PS mitigated the hormetic effect of Cd on worm burrowing behaviour, and this influence was also Cd concentration-related.

Histopathological analyses demonstrated a trend of epidermal and intestinal damages for Cd alone and their co-exposure group with PS or PET. Overall, our results indicate that the bioavailability and toxic effects of Cd could be altered with the presence of PS or PET for *P. aibuhitensis*, which provide new insight for joint ecological risk assessment of legacy and emerging contaminants in coastal environment.

### 3.22.P-Tu361 Ecotoxicological characterization of plastics from four Po River tributaries (Northern Italy)

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The demand of plastics follows a positive trend worldwide, resulting in an increase of plastic production from 365 million tons in 2018 to 391 in 2021. Consequently, a continuous release of these synthetic materials in both marine and freshwater environments is documented. Nonetheless, the effects of plastics to freshwater benthic organisms are poorly studied. Therefore, the aim of this study was to assess the effects of “environmental plastics” collected from four Po tributaries as Ticino, Adda, Oglio and Mincio Rivers (Northern Italy) using *Chironomus riparius* as biological model. Samplings were performed in June 2022 collecting floating plastics with plankton nets (100 µm mesh), and sediments with a Van Veen grab. After plastic extraction by collected samples, the obtained particles were quantified and characterized (shape, size, colour and polymer composition) through the Fourier Transform Infrared Microscope System (µFT-IR). The main results of monitoring campaigns showed a higher contamination of sediments compared to water for all considered rivers, with significant differences ( $p < 0.05$ ) between water and sediments of Adda and Oglio Rivers. We observed a domestic contamination, due to polyester fibers, in water and sediments of Ticino and Adda, and in sediments of Oglio River only, while an industrial pollution, due to polystyrene pellets, was observed in both water and sediments of Mincio and in Oglio River water. Concerning the effect evaluation (chronic toxicity), the characterized particles were used in the exposure of specimens of *Chironomus riparius* following the guideline of Organisation for Economic Cooperation and Development (OECD) number 233. Moreover, a biomarker suite represented by endpoints of cellular stress and neurotoxicity was applied. In addition, we performed the same ecotoxicological tests also using polystyrene beads of 1 µm to compare the toxicity of these standards to those induced by “environmental plastics”.

### 3.22.P-Tu362 Ecotoxicity assessment of hydrophobically-modified and non-modified cationic cellulose originated from Acacia wood

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Cellulose derivatives can be allied tools in the fight against microplastics, encouraging their sedimentation. However, the development of these derivatives requires fine-tuning so that they do not become a strain on the environment. Therefore, this study aimed at physicochemical characterization of these polymers followed by evaluating ecotoxicity of three cationic-modified and one hydrophobically-modified cellulose polymers with different substitution degrees (DS +0.3, +1, +1.8, and DS -1H) in four freshwater trophic level representative species: *Raphidocelis subcapitata* (biomass and growth rate), *Daphnia magna* (mortality), *H. viridissima* (mortality) and *Danio rerio* (mortality, malformation, and heartbeat rate). Suspensions of 0.007%, 0.01%, 0.015%, 0.023%, 0.034%, and 0.051% (w/w), of the cellulose polymers, plus controls, were tested, with 0.01% being the concentration of interest for plastics flocculation. Parallel characterization of the suspensions was also performed. Whenever possible the median lethal and sublethal concentrations (LC<sub>50</sub> and EC<sub>50</sub>) were computed.

All polymers were found to be very toxic for *H. viridissima* (100% mortality at all tested concentrations) and *R. subcapitata*. In *R. subcapitata*, all polymers induced high aggregation and sedimentation of the microalgae cells. Considering the results based on *D. magna* and *D. rerio*, the hydrophobically-modified cellulose polymer (DS -1H) was found to be one of the least toxic polymers for the former and most toxic for the later species. All cationic-modified polymers induced toxicity to *D. magna* in the same order of magnitude ( $0.022\% < LC_{50} < 0.024\%$ ), whilst for *D. rerio* the toxicity ranking of the tested polymers based on mortality was as follows: DS +0.3 < DS +1 < DS +1.8. For cationic polymers with varying substitution degrees, it is hypothesized that low DS impedes complete solubilization in aqueous media, causing interference with biological membranes, whilst high DS leads to strong interaction with the cell wall, potentially causing higher toxicity.

Based on the variety of responses obtained for the four species representing different trophic levels, it is highlighted that the development of eco-friendly methodologies to overcome plastic pollution must be carefully evaluated prior to their marketing, otherwise, they themselves may be a cause for concern in the ecosystems.

### 3.22.P-Tu363 Biodegradable plastics can mitigate plastic pollution? What marine organisms tell us

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Environmental pollution caused by plastic is estimated at millions of tons, mostly fragmented into micro- and nano-plastics with major consequences on the environment and for human health. In particular, the marine ecosystem represents a very strong combination of abiotic and biotic factors and a source of food for people too. The sea characteristics differ greatly from the composting, industrial and laboratory conditions for which the plastic polymers were mostly designed. The study of the environmental and human fate and potential toxic effects of micro- and nano-plastics by a multidisciplinary and one health approach (from the environment to humans) is particularly valuable. To evaluate whether the use of biodegradable polymers can mitigate plastic pollution in the marine environment, we assessed the potential ecotoxicity of microplastics, obtained at laboratory scale, from biodegradable and non-biodegradable polymers. The biological response was evaluated on marine organisms with different physiological, behavioural and feeding characteristics: bacteria, algae, rotifers, crustaceans, mussels and sea urchins. Preliminary results highlighted morphological malformations in sea urchin embryos exposed to polylactic acid, which at a molecular level are due to variations in gene expression levels relating to cellular processes such as skeletogenesis, differentiation and development, stress and detoxification response. Investigations are currently underway on the others and an integrated assessment of the ecotoxicological battery will help to establish whether biodegradable polymers are less toxic than non-biodegradable ones.

### **3.22.P-Tu364 Impacts of polystyrene on growth and nutritional profile of standard freshwater species**

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The proliferation of plastic waste, including the more recent emergence of microplastics in aquatic systems, has surpassed our ability to manage them effectively. Due to their ubiquity, isolated or combined toxicity, persistence, and high densities, microplastics have become a global concern. Polystyrene stands out as one of the most prevalent polymers in the environment, and as a result, it has been extensively studied in terms of toxicity. However, the impacts of polystyrene on freshwater systems and biochemical parameters (e.g., fatty acids profile) have been overlooked. Fatty acids (FAs) are essential molecules for the maintenance of organisms' physiological functions acting also as bio-indicators of stress conditions. Hence, the aim of this study was to evaluate the effects of 1 µm polystyrene microspheres on the growth, mortality/immobilization and nutritional profile (i.e., FAs) of the four freshwater standard organisms (a microalgae *Raphidocelis subcapitata*, a macrophyte *Lemna minor*, a crustacean *Daphnia magna* and an insect larvae *Chironomus riparius*). Each bioassay included four concentrations of microplastics: 50, 500, 5,000 and 50,000 p L<sup>-1</sup> – for planktonic species; and 7, 70, 700 and 7,000 p g<sup>-1</sup> – for benthonic species. Results suggested that microplastics did not affect the mortality/immobilization and the growth of the four freshwater organisms. In fact, when exposed to polystyrene the microalgae growth was even stimulated. Ongoing analysis of fatty acid abundance and composition will provide further insights. We anticipate that our study will shed light on the complex interactions between polystyrene microplastics and freshwater organisms, emphasizing the need for a comprehensive understanding of their implications at a sub-individual level.

### **3.22.P-Tu365 Fate and effects of an environmental realistic mixture of microplastics in freshwater microcosms**

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The majority of studies assessing the effects of microplastics (MPs) in freshwater ecosystems have been performed using reference materials of a certain size, shape and polymer type. In order to improve realism in the aquatic risk assessment of MPs, experiments should characterize the actual exposure pattern of environmentally realistic mixtures of MPs in water and sediment compartments. The aims of this study were (1) to determine the aquatic fate and the water-sediment fluxes of a MP mixture with different shapes and polymers, (2) to characterize the internal concentration of the MPs in two invertebrate species with different biological traits, and (3) to assess their long-term effects on lethal and sub-lethal endpoints. The MP mixture was selected based on the most common polymers ((high density polyethylene (HDPE), polypropylene (PP) and polyester (PES)) and shapes (fragments and fibres) found in freshwater ecosystems with a particle size range between 36 and 3887 µm. The test species were *Lymnaea stagnails* and *Lumbriculus variegatus*. Four MP concentrations were tested ranging from 0.1 to 10 g/kg of sediment. MP ingestion and size as well as mortality, reproduction, and weight or length were assessed in both species to determine No-Observed Effect Concentrations (NOECs). Additionally, samples of the surface water, column water and sediment were collected after a stabilization period and after the chronic test to assess concentrations in each compartment and water-sediment fluxes. Most MPs were retained in the sediment and fluxes of MPs occurred from the floating phase to the sediment, reducing the percentage of total MPs in the floating phase from 3.3% to 0.3% and increasing in the sediment from 96% up to 99%. Uptake showed that ingested PES fibres and PP fragments had a significantly smaller size than the initial mixture. Both species ingested mostly MPs fragments below 250 µm and fibres below 500 µm. Significant reductions were found for shell length and number of egg clutches of *L. stagnails* with a NOEC of 0.5 g/kg and 1 g/kg, respectively. A significant reduction was also found in the number of alive adults of *L. variegatus* with a NOEC of 0.1 g/kg.

These concentrations are found in freshwater sediments in some industrial and urban areas; therefore, this study suggests that realistic concentrations can pose a risk to pelagic and benthic species in freshwater ecosystems.

### **3.22.P-Tu366 Sediment Matters as a Route of Microplastic Exposure: A Call for More Research on the Benthic Compartment**

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Microplastics (MPs) are ubiquitous in marine environments. Here, most MPs are expected to sink and accumulate in the sediment owing to factors like the density of the MP polymer and various environmental processes, such as biofouling and fecal pellet packaging. However, a discrepancy emerges between the expected accumulation of MPs in the sediment and the focus of most MP effect studies, which typically involve pelagic species and employ water-only exposures. To quantify this critical knowledge gap, we conducted a systematic literature search to address pivotal aspects of MP pollution in marine environments, specifically linking ingestion and adverse effects of sediment-associated MPs on benthic invertebrates at various levels of biological organization. An extensive literature search was conducted using various keyword combinations such as "microplastic," "plastic microparticles," or "microfibers" combined with "toxicity," "effect," "impact," or "ecotoxicity," and further associated with "sediment" or "benthic." Specifically, we exclusively included articles that investigated exposure through sediment, (*i.e.*, sediment-associated MP exposure). Studies concentrating solely on ingestion, disregarding associated effects, and those where contaminants were linked to the exposure were systematically excluded from the analysis. The analysis identified 10 effect studies that involved MP exposure via sediment, with the majority concentrating on sub-organismal and organismal effects. Consequently, there is a notable lack of studies that specifically address the effects on benthic invertebrates and those exploring impacts at higher levels of biological organization. To achieve a thorough understanding of the consequences of MPs in marine ecosystems, it is crucial to conduct additional research. Recommended strategies to guide and organize this research include emphasizing the significance of sediment as a pathway for MP exposure, conducting studies with environmentally realistic exposure concentrations and durations to capture potential long-term effects, and focusing on the impacts of MPs at the population and community levels to achieve a comprehensive understanding of their ecological implications in marine ecosystems. Implementing these recommendations will advance our understanding of MP pollution and its implications.

### **3.22.P-Tu367 Multilevel biological responses of *Daphnia magna* exposed to nano-size engineered polystyrene and polyvinyl chloride plastics**

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A growing number of studies have reported the toxic effects of nanoplastics (NPs) on organisms. However, the focus of these studies has almost exclusively been on the use of polystyrene (PS) nanospheres, while the effect evaluation of other polymers, even more present in the market, is still unexplored.

The present study aims to determine if different NP polymers exhibit comparable toxicity, through the evaluation of the sublethal effects on *Daphnia magna* of two different NP polymers: PS with an average size of 200 nm, and polyvinyl chloride (PVC) with a comparable mean size distribution between 50 and 350 nm. The occurrence of adverse effects at different biological levels, induced by both short-term and long-term exposure, has been assessed. Five environmentally relevant concentrations, from 2.5 to 250 µg/L, were tested for each polymer for an exposure time of 48 h. Additionally, a concentration of 10 µg/L was tested for a longer period of 21 days. After the short exposure of 48 h, NP effects were assessed at the biochemical level by investigating the amount of reactive oxygen species and the activity of the antioxidant enzyme catalase, and at the individual level by evaluating the swimming behavior. We have further investigated the potential role of the NP polymer in the onset of morphological, physiological, survival, reproductive, and behavioral alterations after 21 days of exposure.

The results of the short exposure show that PVC-NPs, in contrast to PS-NPs, can pose sublethal effects on *Daphnia magna* both at the biochemical and individual levels, while those of the long exposure reveal that PVC-NPs can lead to an increase in both the molting behavior and body size of the exposed individuals. Moreover, PVC-NPs affect the reproduction of *Daphnia magna*, causing a retard on the day of the first brood and a general decrease in the number of broods and offspring.

Our research suggests that during both the exposure periods PVC-NPs, in contrast to PS-NPs, can pose sublethal effects on *Daphnia magna*. The role of particle size on the observed impacts cannot be disregarded, given that PVC-NPs exhibited a wider size range than PS-NPs, spanning from 50 to 350 nm. Despite this, it is essential to consider the chemical composition of

the plastic polymers in addition to particle size. Since PVC appears to be more hazardous than PS, we therefore suggest continuing to explore the role of the polymer type in the onset of NP hazard.

### **3.22.P-Tu368 Impact of Weathered and Virgin Polyethylene Terephthalate Nanoplastics on Growth Dynamics and the Production of Extracellular Polymeric Substance (EPS) by Marine Algae**

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In context of the ever-increasing plastic waste accumulation in the marine environment, it is important to understand the interaction between microalgae and nanoplastics (NP), and the role of extracellular polymeric substances (EPS) within this. EPS production is a known algal stress response, and its adhesive properties may induce aggregation of both algae themselves and particles, which in turn may affect ecological and hydrodynamic processes such as trophic transfer of nanoplastics or the vertical transport. Here, the impact of fragmented, polydisperse virgin polyethylene terephthalate (PET,  $D_{\text{average}} = 1400$  nm) and weathered polyethylene terephthalate (PET,  $D_{\text{average}} = 680$  nm) on algae growth and the production of EPS was studied by exposing the marine microalgae *Rhodomonas salina* to low NP concentrations (10, 100 and 1000 and 10000 NPs ml<sup>-1</sup>) for 12 days. A positive control with kaolin was included to detangle particle effects from plastic effects. Baranyi parametric growth-models were fit to the data to analyze growth-dynamics. Exposure to weathered PET, virgin PET and kaolin resulted in an initial increased growth rate, followed by significant decreases in algae population density. At low concentrations, the effect was independent of the particles' nature (natural or anthropogenic) or age (virgin or weathered). At high exposure concentrations, the plastic particles caused significantly higher decreases in population density, and the effects were amplified as the particles weathered. The effects on growth of weathered PET were combined with significant increases in cellular EPS production. This suggests that algae exhibit an increase in EPS-production as a stress response. This raises questions about the toxicity mechanisms of NPs in low concentrations, and hints towards the role of EPS production as a defence mechanism, which changes the energy budgets, with less energy allocated to growth. This study underscored the intricate interactions between particle types, age and concentrations, and their distinct impacts on algae density, growth inhibition and EPS production.

### **3.22.P-Tu369 Impact of secondary biodegradable nanoplastics on the freshwater microalga *Chlamydomonas reinhardtii***

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During the last years, there has been a transition from conventional plastics to biodegradable alternatives. Although many works have been published about the ecotoxicological effects of conventional and primary microplastics and nanoplastics (NPs), limited knowledge exists regarding the impacts of biodegradable plastics, and the secondary NPs formed by their fragmentation. This study focuses on elucidating the toxicological effects of secondary NPs obtained from PBAT (Polybutylene adipate co-terephthalate), a biodegradable plastic commonly used in agriculture mulching, on the green microalga *Chlamydomonas reinhardtii*, a key model organism in freshwater ecosystems due to its role as a primary producer. Secondary PBAT nanoplastics (PBAT-NPs) were obtained by mechanical breakdown and photooxidation of pristine PBAT microbeads. For the physicochemical characterization of secondary PBAT-NPs, hydrodynamic size, surface charge, morphological identification, chemical identification, and final concentration were measured and conducted in ultra-pure water using dynamic light scattering (DLS), electrophoretic light scattering (ELS), scanning electron microscope (SEM-EDX), Fourier transform infrared spectroscopy (FTIR) and dry weight, respectively. To investigate the effects of the secondary PBAT-NPs, *C. reinhardtii* was exposed to different concentrations of secondary PBAT-NPs and the following parameters were assessed: cellular growth, photosynthetic pigments contents and fluorescence measurements, photosynthesis, ROS generation, membrane potential, intracellular pH, and metabolic activity measurements. Results showed that secondary PBAT-NPs can heteroaggregate with *C. reinhardtii* and decrease the microalga photosynthesis, trigger ROS overproduction, and cause membrane depolarization. Transcriptomic analyses by RNA-Seq will also be performed. Taken together, this study aims to unravel the underlying mechanisms governing the potential biological impact of secondary biodegradable NPs, evaluating the risk of this novel alternative to conventional plastics in freshwater ecosystems.

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### **3.22.P-Tu370 Influence of Benzo[a]pyrene on the Toxicity of Polystyrene Nanoplastics to Marine Microalgae *Isochrysis galbana***

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Due to the widespread use of plastics since the 1940s, their annual production continues to rise. Consequently, their release into aquatic environments is also increasing. Once in the environment, plastics can undergo fragmentation into micro (1  $\mu\text{m}$ -1 mm) and nanoplastics (NPs,  $<1 \mu\text{m}$ ) due to physical, chemical, and biological processes. In addition, NPs can transport environmental pollutants and enhance their bioavailability to organisms. To enable a more comprehensive understanding of the effects of NPs on primary producers and their role as carriers of potentially harmful contaminants, the aim of this study was to determine the toxicity of NPs alone and with the model polycyclic aromatic hydrocarbon benzo[a]pyrene (B[a]P) on the marine microalgae *Isochrysis galbana*. *I. galbana* cultures were exposed to 0.5  $\mu\text{m}$  polystyrene (PS) NPs (0.0078  $\mu\text{g/L}$ -778  $\mu\text{g/L}$ ) or to 50 nm PS NPs (6.86E<sup>-07</sup>-E<sup>-01</sup>  $\mu\text{g/L}$ ) with or without 1 mM B[a]P. These NP concentrations are equal to 1E<sup>02</sup>-1E<sup>07</sup> particles/mL. Alterations in microalgae growth (average specific growth rate, ASGR), cell viability and reactive oxygen species (ROS) production were investigated. After 72 h, *I. galbana* exposed to 50 nm NPs exhibited no significant changes in growth. In contrast, 0.5  $\mu\text{m}$  NPs markedly reduced ASGR across all tested concentrations, except for E<sup>+07</sup> part/mL. Cell viability was significantly reduced after exposure to most concentrations tested while ROS production was not altered. Though B[a]P alone decreased microalgae growth, co-exposure to NPs and B(a)P did not alter ASGR and viability of microalgae, except at the highest concentrations tested (1E<sup>06</sup>-1E<sup>07</sup> particles/mL), which resulted in a significant decrease in both endpoints. These results can be related to the increase in ROS production observed at the highest NPs concentrations tested with B[a]P, while a significant decrease was observed in cells exposed to B[a]P alone. Overall, the combination of B[a]P and NPs exhibited increased toxicity to *I. galbana*, particularly at high NP concentrations, compared to NPs alone. Though further analyses are needed, the results obtained could be explained by the different behavior of NPs in the exposure media, concentrations tested and the presence of B[a]P. Work funded by Spanish MINECO NACE project (CTM2016-81130-R), MICINN FIERA project (PID2021-128600OB-I00, MCIN/AEI/ 10.13039/501100011033 and “ERDF A way of making Europe”) and the Basque Government grant to consolidated research groups (IT1743-22).

### 3.22.P-Tu371 Histopathological Analysis of Mussels *Mytilus galloprovincialis* after Foodborne Exposure to Three Sizes of Polystyrene Nanoplastics

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Given the widespread occurrence of polystyrene (PS) micro and nanoplastics in the marine environment, it is important to determine their potential adverse effects on representative sentinel marine species as mussels. Foodborne exposure is more environmentally realistic than waterborne exposure in mussels but it has received less attention. The aim of this work was to assess the histopathological effects of foodborne exposure to PS NPs of different sizes on marine mussels, focusing on inflammatory reactions involving hemocytes, since MPs and NPs have been reported to produce inflammatory responses. Mussels *Mytilus galloprovincialis* were dietarily exposed through algae *Isochrysis galbana* to PS NPs of 50, 200 and 1000 nm at nominal low (10<sup>3</sup> NPs/mL) and high concentration (HD) (10<sup>8</sup> NPs/mL for 50 and 200 nm NPs and 10<sup>6</sup> NPs/mL for 1000 nm NPs). Exposures took place for 7 days in duplicate and two independent experiments were carried out per NP (E1 & E4 for 50 nm, E2 & E5 for 200 nm and E3 & E6 for 1000 nm PS NPs). All exposures were developed along 3 weeks in September-October 2022 (E1&E2, E3&E4 and E5&E6), in order to minimize the influence of season on studied parameters. High prevalences of hemocytic infiltration, fibrosis and atrophy of the digestive tubule epithelium were detected in the digestive gland. However, these could not be related to the foodborne exposure to NPs because no significant differences were found between exposed and control mussels. Observed alterations could be due to the presence of parasites and/or to the stage of gametogenic development, since mussels were mainly in advanced gametogenesis in E1&E2, spawning in E3&E4 and mature in E5&E6. In the gonad, prevalences of the different alterations were generally lower than in the digestive gland and they could not be related to NPs exposure either. In conclusion, the reproductive status appeared to contribute more to observed alterations than PS NP exposure. It remains to be determined whether longer term exposures could result in more severe histopathological alterations, independent of the physiological condition. Further work is required on environmentally realistic NPs that might pose additional risks compared to pristine ones. \*Work funded by the EC CAS NANOPLASTICS project (DG JRC), the Spanish MICINN FIERA project (PID2021-128600OB-I00, MCIN/AEI/ 10.13039/501100011033 and “ERDF A way of making Europe”) and the Basque Government grant to consolidated research groups (IT1743-22).

### 3.22.P-Tu372 Microplastics in Pelagic Fish and Surface Water from the St. Lawrence River and Estuary, Canada

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In Canada, the St. Lawrence River and Estuary form an important aquatic system that links the Great Lakes to the Atlantic Ocean and is a vector of emerging contaminants from the land to the ocean. Very limited information is available on microplastics (MPs) contamination in this system, particularly for pelagic fish. To determine the risks that MPs may pose to wildlife and the ecosystem, it is crucial to examine their distribution and fate in the environment. This study aims to investigate the exposure and tissue distribution of MPs in pelagic fish of ecological and economic importance from the upper St. Lawrence River to the fluvial estuary area. For the upper St. Lawrence River, northern pike (*Esox lucius*) (n=40) and its potential prey, the yellow perch (*Perca flavescens*) (n=10), as well as surface water (n=14) were sampled upstream and downstream of Montreal's primary wastewater treatment plant (WWTP). For the freshwater portion of the estuary, walleye (*Sander vitreus*) (n=30), and its potential prey rainbow smelt (*Osmerus mordax*) (n=15) and white perch (*Morone americana*) (n=10), as well as surface water (n=9) were sampled around Québec City. The analysis of MPs was conducted in the gastrointestinal tract (GIT) and the gills of these fish species to elucidate both active and passive ingestion of MPs. Preliminary results showed that the abundance of MPs in northern pike was  $1.59 \pm 0.37$  (mean number of particles  $\pm$  SE) in GIT and  $2.71 \pm 0.42$  in gills, which was not significantly different from yellow perch ( $1.0 \pm 0.37$  in GIT and  $0.67 \pm 0.22$  in gills). The higher number of MPs in gills of pike suggests greater passive ingestion of MPs. The size of MPs was in the range 640 to 1310  $\mu\text{m}$  for the analyzed fish samples. Fiber was the dominant shape of the observed MPs in fish, followed by fragments. The most common color of MPs in fish was blue followed by black. In addition, the abundance of MPs in the samples collected downstream of the WWTP was not significantly different from the upstream samples, suggesting that the WWTP is not a significant source of MPs in downstream water and fish around Montreal. Preliminary polymer identification of particles (n=43) using  $\mu\text{FTIR}$  indicated the presence of polyethylene, polyester, nylon, cellulose, and rayon in northern pike tissues. Analysis of the samples from the estuary area is in process and preliminary results will be presented in this poster.

### **3.22.P-Tu373 Combined Ecotoxicity Effects of Nanoplastics and the Antibiotic Sulphamethoxazole on the Free-Floating Aquatic Plant *Lemna major***

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The exponential growth of plastic production and the resulting contamination of all ecosystems are hallmarks of the Anthropocene. Once exposed to environmental factors, the plastic debris gradually degrades into micro- (1 to 5,000  $\mu\text{m}$ ) and nano-scaled (< 1  $\mu\text{m}$ ) particles. In particular, owing to the large surface area per unit mass, nanoplastics (NPs) show a considerable higher adsorption capacity than the original bulk material, thus acting as vectors for harmful organic contaminants with underestimated ecotoxicological effects on the aquatic biota. The widespread co-occurrence of NPs and pharmaceuticals (including antibiotics), is an urgent issue that requires immediate attention.

The present study focused on the ecotoxicity of polystyrene NPs and the antibiotic sulfamethoxazole (SMX, commonly used for urinary and respiratory infections). Different NPs and SMX concentrations (from 100 to 5 mg/L) alone or in mixture were tested to assess their ecotoxicological effects on *Lemna major* (ISO 20227:2017), a free-floating aquatic plant with well-known contaminant accumulation and purifying capabilities. EC50 values (for each contaminant and for mixture) and chlorophylls content in plants were determined. Flow cytometry was used for NPs quantification and characterization and for assessing microbial cell number in the mineral growth media. Main results showed that a correlation between contaminant concentration and effects were observed. An additive effect of NPs and SMX seems affect plant development, although further studies are in progress.

### **3.22.P-Tu374 Ecotoxicological impact of secondary nanoplastics from pristine and recycled low density polyethylene plastics**

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The rise in recycled plastics usage offers a potential solution to reduce the environmental accumulation of plastic waste. However, it is currently unknown whether these plastics generate particles in the nano-range and whether these nanoplastics (but also additives) may trigger a potential threat to the environment. This study focuses on assessing the toxicological effects of secondary nanoplastics derived from both recycled and non-recycled low-density polyethylene (LDPE) plastics on representative organisms in the aquatic ecosystems. Three model organisms were selected: The green microalga *Chlamydomonas reinhardtii*, the freshwater plant *Spirodela polyrhiza*, along with the water flea *Daphnia magna*. Secondary nanoplastics were obtained through mechanical fragmentation of LDPE microplastics. A comprehensive physicochemical characterization has been conducted to verify the presence of secondary nanoplastics in both types of plastics through several techniques such as dynamic and electrophoretic light scattering, scanning electron microscopy and Fourier transform infrared spectroscopy. The three selected organisms were exposed to increasing concentrations of secondary nanoplastics to evaluate the biological effects of secondary nanoplastics and released by-products. Additionally, mechanistic studies will be conducted to assess potential impacts on specific stress pathways. This study contributes to the assessment of potential toxicity associated



with recycled plastics, specifically addressing the impact of secondary nanoplastics on aquatic organisms, and enhances our overall understanding of the environmental implications of plastic recycling.

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### **3.22.P-Tu375 Trophic Transfer Of Contaminated And Uncontaminated Microplastics: Regeneration Study Evaluated In Planarian Sp.**

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Microplastics (MPs) are particles (1µm to 5mm size) that can transport and transfer chemicals such as the insecticide chlorpyrifos (CLP) producing adverse effects on biota. However, information on the trophic transfer of contaminated and uncontaminated MPs is scarce in fresh water organisms such as planarian spp. The objective of this study was to evaluate trophic transfer, permanence and potential effect of MPs on the regeneration of planarian sp. fed with *Daphnia magna* previously contaminated with uncontaminated MPs of polyethylene (27-32 µm) and MPs contaminated with CLP. Observations of entire planarian sp. were done using stereomicroscope, detecting the presence of MPs by fluorescence. After a second feeding, planarians were decapitated. The effect and permanence of MPs in both segments was recorded from day 4 to day 7, and day 21. Presence of uncontaminated and contaminated MPs were detected even up to day 7 post decapitation. Ocelli appeared after 5 days in all treatments except in those planarians fed with daphnias exposed to uncontaminated MPs, in this treatment a delayed generation of ocelli was observed compared to de control. Presenting one ocellus on day 7 post decapitation, furthermore the second ocellus appeared asymmetrically in size and position on day 21. Head regeneration was significantly affected in planarians fed with daphnias exposed to contaminated MPs compared to the control ( $p < 0,05$ ). This study demonstrate that MPs can be transferred to planarians through their prey, inducing alterations during the regeneration process. results also shows that uncontaminated MPs can induce adverse effects, probably due to release of contaminants added during manufacturing of the plastic. In addition, this study indicates that MPs can act as vector of organic pollutants such as CLP. However, more trophic transfer studies are required to understand potential adverse effects of MPs on fresh water ecosystem. Thanks to FONDECYT INICIACION N° 11180466.

### **3.22.P-Tu376 Possible trophic transfer of polystyrene microspheres from *B. calyciflorus* to its predator *A. brightwelli***

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Microplastics (MP) pollution is one of the biggest threats of our ecosystems, both, terrestrial and aquatic systems. Especially in aquatic ecosystems, MP can affect many species, because animals can mistakenly ingest MP as food items. While the potential for ingestion, accumulation, and the effect in selected organisms is well documented, the transfer and biomagnification over multiple trophic levels are less well studied.

Here we studied potential bioaccumulation and biomagnification of microplastics within limnetic zooplankton. Therefore, we used the herbivorous filter feeder *Brachionus calyciflorus* (Rotifera) and its predator *Asplanchna brightwelli* (Rotifera), as a common predator-prey pair. First, we experimentally exposed *B. calyciflorus* to MP (1, 3, and 6 µm in diameter separately) and quantified the time-dependent excretion of MP. Second, we fed the predator with MP-exposed *B. calyciflorus* recorded the prey ingestion and the potential retention of MP.

We found that *B. calyciflorus* excreted more than 60 % of the MP within 30 minutes suggesting limited long-term uptake. When *B. calyciflorus* with MP was fed to *A. brightwelli*, MP was taken up by the predator but almost completely excreted within 24 hours. We found no clear evidence for the long-term retention of spherical MP in our predator-prey system.

### **3.22.P-Tu377 Characteristics of microplastics ingested by *Acartia* spp. in two enclosed bay environments of Korea**

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The ubiquitous presence and persistence of microplastics (MPs) in various environments have been considered a matter of serious concern due to an increasing threat to ecosystems and even human being. Marine copepods have been traditionally considered the interlink between primary producers and higher trophic levels, although recent studies revealed that most zooplankton are usually opportunistic omnivorous feeders as potential consumer of MPs. We investigated the characteristics of MPs ingested by *Acartia* spp. in the Yeolja Bay and Jinhae Bay of the Korea. The numerical and relative abundances of *Acartia* spp. ranged from 122 to 6,710 individuals (ind.) m<sup>-3</sup> and from 0.29 to 0.97, respectively. Microplastics consumed by zooplankton ranged 0 to 0.06 particles ind.<sup>-1</sup> with 70% occurrence rate. Fragments are the most common type of ingested microplastics that showed the particle size between 0.03 and 0.13 mm. The microplastic abundance consumed by zooplankton ranged from 1.9 to 67.1 particles m<sup>-3</sup>. This is the preliminary study to understand an important route via which microplastics enter the food web, posing a risk to higher predators and potentially human health. Therefore, microplastic distributions of seawater, zooplankton, and higher trophic organisms should be further analyzed to understand the accumulation pattern of microplastics along food chain.

### 3.22.P-Tu378 Transfer of Different Size Microplastics Throughout Marine Environmental Compartments and Their Fate in Organisms

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Microplastics (MPs) have been documented in all marine environmental compartments and have been identified as a growing hazard to marine biota. This study sought to analyse the effect of size of polystyrene (PS) MP in the transfer among different environmental compartments (biota, water and sediment), and its influence in the accumulation, localization, and depuration dynamics in the target tissues of *Mytilus galloprovincialis*, *Hediste diversicolor*, and *Palaemon serratus*. Organisms were exposed to an experimental microcosm with two different set ups (1) 1/5 treatment with 1 µm MPs spiked in seawater and 5 µm MP spiked in sediment and (2) 5/1 treatment with 5 µm and 1 µm MP spiked in seawater and sediment, respectively. Both compartments were spiked with  $1.5 \times 10^7$  total MPs. Organisms went for 5 days exposure and 14 days depuration period, and were then subjected to procedures for both quantitative (alkaline digestion and filtration) and qualitative (cryosectioning) examination under fluorescence microscope. All species accumulated MPs spiked in the water column and sediment irrespective of their habitat, and MPs appeared to transfer vertically by the influence of biota in relation to particle size. Mussels showed a higher accumulation of MPs spiked in the seawater column with a trend of increased the presence for bigger particles. Particles were found in distinct parts of the digestive tract (lumen of the stomach, intestine) and gills of mussels. Mussels effectively eliminated MPs after depuration, with a significant reduction for smaller particles. Polychaetes accumulated a comparable amount of particles that spiked in the water column and sediment regardless of particle size, being these MPs identified predominantly in the hindgut. After depuration, a considerable increase of 5 µm MP spiked in sediment was noted for polychaetes. Shrimps accumulated both size MPs, but more abundantly bigger particles which imply size specificity, being seawater the route of entry. MPs were mainly localized in the digestive tract and the tegument and pereopods. No discernible reduction in MP elimination was seen after depuration. This work has enabled to understand the influence of size in the transfer of MPs between different environmental compartments and their fate in target tissues of organisms with different feeding behaviors. Funded: Basque Government (IT1743-22, IT-1446-22) and MICINN (PID2020-118685RB-I00).

### 3.22.P-Tu379 Greenhouse Plastics as Vectors for Antibiotic Resistance Genes and Pathogens

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Plastic waste is a critical environmental concern particularly in the context of greenhouse agriculture, as greenhouse agriculture depends on hard to dispose plastic products. This turns it into a source of plastic pollution for surrounding ecosystems, a problem compounded by the fact that plastics can be colonized by microorganisms. Greenhouse agriculture plastic waste could thus act as a vector for pathogens and antibiotic resistance genes (ARGs), facilitating their spread from greenhouses to surrounding environments. This study aimed to investigate the presence of both plant and human pathogens, as well as ARGs and mobile genetic elements (MGEs) in the region of Almería, which harboured the highest concentration of greenhouses globally by 2018. The investigation was conducted across three distinct sampling sites: greenhouses; an unprotected area with substantial pollution close to the greenhouses; and the protected natural reserve *Punta Entinas-Sabinar*, located kms away from the greenhouses. Within each sampling site three replicates of soil (collected from the surrounding environment around plastics) and greenhouse plastic waste were sampled. This comprehensive approach allows for a detailed assessment of the potential environmental impact and spread of pathogens, ARGs and MGEs derived from greenhouse operations to surrounding areas. Sampling sites were chosen based on the local greenhouse plastic waste's life cycle: some reaches the protected natural reserve directly while other is dumped in the unprotected area and eventually ends up in the natural reserve. The presence of five human (*Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Salmonella* spp.) and five plant pathogens (*Alternaria* spp., *Botrytis* spp., *Sclerotinia* spp., *Sclerotium cepivorum* and *Oidium neolycopersici*) was studied using quantitative PCR. Resistome analysis was performed by way of SmartChip Real-Time PCR System. Results showed a high diversity of ARGs and MGEs in greenhouse plastic waste, higher than its surrounding soil in two thirds of the samples. The plant pathogen *Botrytis* spp., 18 ARGs and one MGE were only detected in plastic samples in the natural reserve and unprotected area and in both sample types in the greenhouse samples, suggesting that greenhouse plastic waste could have acted as a vector for pathogens, ARG and MGE alike from greenhouse soil through greenhouse plastic waste to the unprotected area and natural reserve.

### 3.22.P-Tu380 Unravelling the ecocorona using a proteomics approach

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Upon entering the environment, the surface properties of pristine plastics are rapidly altered due to interactions with exogenous biomolecules, contaminants, and even microbiota, which ultimately alter their ecological impacts. When present in biological fluids or high protein environments micro(nano)plastics bind with proteins which form a protein corona around the particle. Although a significant body of literature exists on protein corona formation on nanomaterials, less is known about how physicochemical characteristics of microplastics may influence protein corona formation in the environment. This study utilises

quantitative proteomics to quantify protein binding to pristine and leached microplastics to understand fundamental process driving ecocorona formation. Pristine polyethylene (PE) beads (50 and 500 $\mu\text{m}$ ), polyamide (PA) fibres (100 $\mu\text{m}$ ), polyethylene terephthalate fibres (500 $\mu\text{m}$ ) and fragments (<300 $\mu\text{m}$ ), as well as pristine and leached textile microfibrils comprised of PET, recycled PET, PA or cotton were incubated for 24 hours in bovine serum albumin solution (2mg mL<sup>-1</sup>) to form a corona. Protein adsorption to microplastics was dependant on particle surface area to volume ratio but only when additives were not present. For environmentally relevant textile microfibrils, cotton microfibrils adsorbed significantly more protein than synthetic microfibrils. Fourteen-day aqueous leaching increased the surface charge of all microfibrils, however only PA fibres sorbed significantly higher protein on the leached fibres compare their pristine counterparts. Overall, the presence of chemical additives in microplastics was seen to strongly influence protein corona formation. As the majority of microplastics in the environment are generated from consumer products, chemical additives are likely to play a significant role in corona formation in the environment. Further, proteomic methods developed for this study can be used to quantify the ecocorona formed on microplastics in environmental compartments, facilitating greater understanding of microplastics toxicology.

### **3.22.P-Tu381 Environmental DNA adsorption onto polymers in environmental waters: A comprehensive study on the protection of adsorbed DNA**

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In environmental systems, microplastics (MPs) (1  $\mu\text{m}$  – 5 mm) are found alongside environmental DNA (eDNA). eDNA is genetic material shed by organisms through decayed cells, serving as a valuable tool for non-invasive biodiversity assessments and aiding conservation efforts by enabling species presence monitoring. eDNA that sorbs onto particles have been shown to be longer-lived than when in suspension, likely because of protected from enzymatic degradation. Consequently, measuring adsorbed eDNA on MPs could provide insights into plastics origin and environmental transport pathways. For this, better understanding of binding kinetics, adsorption extent and mechanisms of eDNA onto MPs is needed, including water chemistry parameters influencing adsorption. Furthermore, assessing the longevity of MPs bound eDNA when exposed to a restriction enzyme is crucial when considering its potential for source tracking. In this study, interactions between a model linear eDNA (O.Keta salmon sperm DNA) and thin polymer films (PET, PE) were investigated. Adsorption experiments using Quartz-Crystal Microbalance with Dissipation monitoring (QCM-D) allowed to investigate adsorption of small amounts of eDNA over time and to access viscoelastic parameters such as thickness, viscosity and shear modulus of adlayers. Variations in water chemistry, including ionic strength (similar to marine and very hard freshwater) and cations concentrations and compositions (Na<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>) were considered to understand adsorption mechanisms. The protection of adsorbed eDNA against enzymatic degradation using DNase I as model restriction enzyme allowed us to quantify the longevity of surface-bound eDNA. Cation-bridging induced by divalent cations was shown to be the main component driving adsorption, with Ca<sup>2+</sup> demonstrating a more pronounced effect on the adsorption process compared to Mg<sup>2+</sup>. Between the two polymer surfaces, PET exhibited stronger affinity and faster adsorption, likely because of surface chemistry. However, eDNA was only partially protected from enzymatic degradation on PET whereas PE exhibited full protection, either because of surface chemistry or eDNA surface coverage. This study provides first insights into adsorption interactions between eDNA and polymer surfaces, allowing us to assess the effectiveness of MPs source tracking through eDNA measurement

### **3.22.P-Tu382 Diversity of marine bacteria growing on leachates from virgin and weathered plastic: insights into potential degraders**

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Plastic debris in the ocean releases chemical compounds that can be toxic for marine fauna and which may have an impact in the marine carbon cycle. This leaching process is enhanced by sunlight radiation. It was recently found that some marine bacteria are able to degrade such leachates, but information on the diversity of these bacteria is mostly lacking. Here, we will show the results of a study in which marine bacteria were grown on plastic leachates to search for potential degraders. Two types of leachates were generated using new low density polyethylene (LDPE) and a mix of naturally weathered plastic, collected from beach sand, which were introduced in seawater. Some treatments were exposed to sunlight degradation and others were kept in the dark. The amount of dissolved organic carbon in the leachates and its fluorescence signal were explored. We also analyzed the bacterial diversity growing on the plastic leachates. We used a combination of Catalyzed Reporter Deposition-Fluorescence In Situ Hybridization (CARD-FISH) and BioOrthogonal Non-Canonical Amino acid Tagging (BONCAT), and 16S rRNA gene amplicon sequencing to analyze bacterioplankton-groups specific activity responses and the identity of the responsive taxa to plastic leachates produced under irradiated and non-irradiated conditions. We found that material released by plastic in seawater presents a characteristic fluorescence signal with the highest intensity in aged plastic leachates. Regarding the bacteria growing on the plastic leachates, we observed that some generalist taxa responded to all leachates, most of them belonging to the Alteromonadales, Oceanospirillales, Nitrosococcales, Rhodobacterales and Sphingomonadales orders. However, there were also non-generalist taxa responding to specific irradiated and non-irradiated leachates. Our results provide information about bacterial taxa that could be potentially used to degrade the chemicals released during plastic degradation into seawater contributing to bioremediation of plastic pollution and its additives.

### **3.22.P-Tu383 Toxicity of Methacrylate Nanoparticles of Partially Bio-Based Pressure Sensitive Adhesives (PSAs) to Zooplankton\***

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Pressure Sensitive Adhesives (PSAs) are instantaneous adhesion materials widely used in tapes and labels for glass or plastic bottles. They are produced by emulsion polymerization as polymeric nanoparticles (NPs) stabilized by surfactants in aqueous dispersions (latex). Nowadays, searching for sustainability, the petroleum-based polymers are replaced with bio-based. Yet, after their life-time, these waterborne polymers easily ended in our oceans, contributing towards increased microplastic pollution. Presence of the surfactants, may affect further their potential toxicity for the aquatic biota. Here, the toxicity of two different latexes of partially bio-based NPs for PSA applications were tested in two zooplankton species: the rotifer *Brachionus plicatilis* and the brine shrimp *Artemia salina*. PSA1 (NP size: 215 nm) was synthesized from a bio-based seed containing methyl methacrylate (MMA) and partially bio-based isobornyl methacrylate (IBOMA) extracted from glucose. For colloidal stabilization, the conventional surfactant alkyldiphenyloxide disulfonate (Dowfax 2A1) was used. PSA2 (NP size: 348 nm), in addition to MMA and IBOMA, contained partially biobased 2-octyl acrylate, which copolymerizes with the zwitterionic monomer 2-(methacryloyloxy ethyl] dimethyl-(3 sulfopropyl) ammonium hydroxide (DMAPS) for colloidal stabilization. In rotifers, significantly increased mortality after 24 h of exposure was achieved at the highest exposure concentrations, 1000 mg/L for PSA1 and 500 mg/L for PSA2. At 48 h, acute effects were recorded at lower concentrations: 250 mg/L for PSA1 and 125 mg/L for PSA2. In brine shrimps, significant effects were observed only at 48 h at concentrations  $\geq 250$  mg/L for PSA1 and at 1000 mg/L for PSA2. The ingestion test revealed sublethal effects at lower concentrations: at 125 mg/L and 62.5 mg/L for PSA1 and PSA2, respectively, in rotifers and at 62.5 mg/L for both PSAs in brine shrimps. Lower values of acetylcholinesterase activity were measured in both species exposed to PSAs compared to control individuals while effects on catalase activity did not show a clear trend. In summary, acute and sublethal toxicity of these formulations was detected only at very high and likely, non-environmentally relevant concentrations. \*Funded by the Spanish MICIN project FIERA (PID2021-128600OB-I00, MCIN/AEI/ 10.13039/501100011033 and “ERDF A way of making Europe”) and the Basque Government grant to consolidated research groups (IT1743-22).

### 3.22.P-Tu384 Microplastic Exposure Linked with Decreased Lower Trophic Level Fatty Acid Availability and Reduced Yellow Perch Growth in a Large In-Lake Mesocosm Experiment

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Microplastics (MPs), defined as particles with longest dimension 1-5000  $\mu\text{m}$ , pollute all ecosystems globally in a wide range of polymer combinations, sizes, shapes, and with a large variety of chemicals added during plastic manufacturing. It is accepted that some aquatic environments are currently at risk from MP pollution, but few studies have investigated how MPs affect biologically important nutrients like fatty acids in a food web and the potential for indirect, nutritional effects on consumers. To explore how MPs impact aquatic food web processes, among other research questions, we conducted a large, in-lake mesocosm experiment. We exposed aquatic communities, including plankton and yellow perch (*Perca flavescens*), to MPs with and without chemical additives, and monitored fatty acids in the food web. We predicted that MPs would have greater effects on the base of the food web (phyto- and zooplankton), decreasing the amount of polyunsaturated fatty acids (PUFAs) available to yellow perch, reducing PUFA concentrations in their tissues, and potentially limiting growth. Our experiment had three treatments (n = 3 mesocosms per treatment): negative control, MPs (equal parts polystyrene (PS), polyethylene (PE), and polyethylene terephthalate (PET) with chemical additives (e.g., dyes, UV stabilizers, and antioxidants), and MPs without chemical additives. We added MPs at nominal concentrations of 29,240 particles L<sup>-1</sup>. Zooplankton were less dense in total fatty acids and PUFAs in the plastic treatments, despite a lack of difference in community composition, and PUFAs were reduced in the detritus and in the non-additive treatment wall biofilm. Yellow perch grew less in the plastic treatments, likely due to reductions in important dietary fatty acids in lower trophic levels. Although the yellow perch were able to mostly maintain ratios and concentrations of PUFAs in their liver and muscle, the substantial impact on PUFA availability at the base of the food web seems to have been growth-limiting. Our findings highlight the presence of sublethal, indirect effects of MPs and the importance of considering how contaminants act on biological systems beyond the organismal level.

### 3.22.P-Tu385 Biodegradable microplastics: Uptake by and effects on the rockpool shrimp *Palaemon elegans* (Crustacea: Decapoda)

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Ingestion of microplastics can lead to deleterious consequences for organisms, which has been documented in numerous laboratory studies. The current state of knowledge is based on a multitude of effect studies conducted with conventional fossil-based and non-degradable plastics. However, there is a lack of information regarding the effects of novel bio-based and

biodegradable plastics. Biodegradable plastics are considered an alternative to conventional plastics and are rapidly growing in their production. Biodegradable plastics can reach the environment in the same way as conventional plastic, becoming available to marine organisms. This study aims to provide new insights into the uptake and effects of biodegradable microplastics on marine invertebrates. Rockpool shrimp *Palaemon elegans* were offered algal flakes coated with polylactic acid (PLA), polyhydroxybutyrate-co-valerate (PHBV) and conventional low-density polyethylene (LDPE) microparticles. Live observations showed that all different microplastics were ingested. After dissection of the shrimp, less LDPE particles than PLA and PHBV particles were found in the stomachs, indicating a longer retention time of biodegradable microplastics. Presumably, less LDPE particles were ingested or preferentially regurgitated. The ingestion of microparticles from all plastics induced enzymatic activity of short-chain carboxylesterases in the midgut glands of the shrimp. However, only PLA induced enzymatic activity of medium-chain carboxylesterases. *P. elegans* showed no oxidative stress response after ingestion of microparticles, irrespective of polymer type. We conclude that biodegradable plastics might have different effects compared to corresponding conventional plastics due to their longer retention times and increased biodegradability, leading to an enhanced exposure to leaching additives and other harmful substances. Our results highlight the need for further research and risk assessment of the new bio-based and biodegradable plastic materials.

### **3.22.PC The Fate and Effects of Micro- And Nano-Plastics in Relation to Ecosystems**

#### **3.23.A Tire-Road Wear Particles: Analytical Possibilities, Challenges, and Current Knowledge of Environmental Impact**

##### **3.23.A.T-01 Occurrence and Leaching Potential of Tire Rubber-Derived Compounds from Vancouver Road Dust**

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Tire and road wear particles (TRWP) are non-exhaust particles that typically arise from abrasion, including via brake wear, abrasion of tires by road surfaces, wearing of road material, and agglomeration of tire particles with other road dust particles. The emission of TRWPs is a global challenge, in part because these particles have the potential to release toxic substances. For example, a recent study found a tire rubber-derived chemical called 6PPD-quinone (N-(1,3-dimethylbutyl)-N'-Phenyl-p-phenylenediamine-quinone), a transformation product of the ubiquitous tire rubber antioxidant 6PPD, in road runoff samples collected in Seattle and Los Angeles (USA) at concentrations high enough to induce acute mortality of coho salmon (*Oncorhynchus kisutch*). The prevalence of 6PPD and 6PPD-quinone in the environment has since drawn increasing concerns. Measuring TRWPs and 6PPD and 6PPD-quinone near their source (roadways) can help us better understand TRWP pollution and the release of associated chemical contaminants to the environment.

The factors affecting 6PPD-quinone production can be divided into traffic and roadway circumstances, which affect TRWP production; and environmental conditions, which may affect the formation rates of 6PPD-quinone on tire rubber surfaces. We will conduct roadside dust sampling and subsequent leaching experiments over one year from 5 locations: a high-speed road, an on- and off-ramps on a high-grade bridge, a busy traffic intersection, and a less-busy residential street. These experimental results will deepen our understanding of the environmental occurrence and fate of 6PPD and 6PPD-quinone. Specifically, we can learn about (i) seasonal trends of 6PPD and 6PPD-quinone concentrations in road dust, (ii) the effects of road type and traffic on 6PPD and 6PPD-quinone concentration in road dust, and (iii) 6PPD and 6PPD-quinone leachability and potential impacts on coho salmon. Our first two sets of leaching experiments indicate that sunny and warm conditions may favor the generation or leaching of 6PPD-quinone in TRWPs. We will continue to evaluate the impact of traffic conditions or driving behavior on the generation of TRWPs and the impact of ground-level ozone concentration on the production of 6PPD-quinone through the collection of additional road dust samples.

##### **3.23.A.T-02 Analysis of tyre wear and other road-derived microplastics collected from urban roadside via atmospheric deposition sampling**

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Tyre and road wear particle (TRWP) particle emissions contribute significantly to microplastic concentrations in the urban environment. Tyre materials are extremely complex, containing multiple polymer types (natural rubber-NR, styrene butadiene rubber-SBR, and nylon), inorganic fillers (silica, carbon black), crosslinking agents, vulcanization agents, and additives (up to 10%) at various concentrations. Other emissions can include brake wear particles, road surface wear particles, road paint materials, and polymer modified bitumen. Although TRWP account for 30% of the terrestrial microplastic burden, analytical challenges exist for their detection and quantification. To address this gap, environmental samples were collected adjacent to a busy urban road (the Bristol Road (A38) close to the University of Birmingham, UK) via atmospheric deposition across the Spring and Summer 2023, supplemented by use of Go Pro cameras for determination of traffic counts. TRWP contained in these 68 samples were extracted and organic debris digested using an established and robust protocol developed in-house. Analytical methods based on optical microscopy and pyrolysis gas chromatography mass spectrometry (py-GCMS) were developed to characterize and quantify the TRWP isolated from these samples. This dual mode of detection determines the size of microplastics present (optical microscopy) and the concentrations of polymer types present (e.g., NR and SBR by py-

GCMS) as an estimate of the mass of particles recovered. To target tyre wear particles (TWP) specifically, a py-GCMS method was developed. Exploration of the appropriate pyrolysis products to measure TWP was conducted, and 4-vinylcyclohexene (4-VCH) and dipentene (DP) were found to be the best suited markers for SBR and NR polymers, respectively. Utilizing this information, stock polymer reference materials were obtained, dissolved in the appropriate solvents, and serially diluted to obtain calibration standards in the nanogram/microgram range. A range of sample preparation techniques were explored for TWP analysis by py-GCMS, including hydrofluoric acid digestion, accelerated solvent extraction, microwave assisted extraction, and manual grinding of filter substrates for direct analysis. The workflow developed for roadside sampling, extraction of TRWP from filters, and analysis by optical microscopy and Py-GCMS presented here represents a step-change in analysis capability for these hard-to-measure and quantify particles.

### **3.23.A.T-03 Breaking Down and Disentangling the Complexity of Road Runoff – Results from Three Comprehensive Road Runoff Case Studies in Aachen, Germany**

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Recent research recognized road runoff and tire and road wear particles (TRWP) as a pervasive source of pollution, emphasizing, however, knowledge gaps regarding their emissions, environmental effects and sampling standardization. Targeting these gaps, the RoadTox project conducted a thorough investigation into stormwater runoff from heavily frequented roads in Aachen, Germany, from February 2022 to August 2023.

Three distinct road types —federal highway (BAB4), country road (L23), and city road (B57)— were selected based on their daily traffic volumes. Automated sampling of full-stream runoff yielded original, 63µm, and 0.5µm fractions for physico-chemical and ecotoxicity assessments. Physico-chemical analyses included suspended particulate matter (SPM), tire rubber content, polycyclic aromatic hydrocarbons (PAHs), heavy metals, and mineral oils. Ecotoxicity assessments encompassed a comprehensive bioassay battery, covering daphnia, algae, fish embryos, and *in-vitro* assays (µEROD, (anti-)ER/AR and P53 CALUX®).

Our results revealed consistently elevated pollution levels, surpassing EU standards for PAHs and heavy metals. PAH and mineral oil concentrations correlated with SPM, while over 90% of SPM were <63µm, challenging sedimentation-based treatment systems. Targeted and non-targeted screenings identified 30 to >150 chemicals per sample, including prevalent compounds such as Hexa(methoxy-methyl)melamine (HMMM) and diphenylguanidine (DPG), benzothiazole derivatives, and 6PPDQ. Notably, highway samples obtained under freezing conditions contained high salt loads. Overall, acute toxicity varied across test systems, with zebrafish displaying the highest sensitivity, even in diluted samples. Our studies highlighted increased toxicity during winter runoff and prolonged dry periods, supporting the "first flush" hypothesis and first principal component analyses linked toxicity to mineral oil and high molecular weight PAHs.

Despite the complex nature of road runoff, factors such as particle concentration, anti-freezing agents, and hydrocarbon compounds were identified as influential in determining toxicity. However, predicting road runoff toxicity remains challenging, and hence, ongoing research will explore transcriptome-level mechanistic endpoints in zebrafish to support toxicity-driver identification.

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### **3.23.A.T-04 Quo Vadis Tire Wear Leachable? – Suspect Screening for More than 70 Tire Wear Leachables in the Aquatic Environment**

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Tires are a chemically complex and heterogeneous mixture and contain up to 200 different additives. Tire wear particles (TWP), generated while driving, are pollutants themselves, but also serve as a source for a variety of organic substances that can enter the aquatic environment. While multiple of those so-called tire wear leachables have been identified in screening studies, their environmental fate remains mostly unknown. This lack of data hinders the assessment of their environmental impact and potential risks. To narrow this knowledge gap, we performed an environmental screening for over 70 tire-leachables and investigated the role of wastewater treatment plants (WWTPs) as entry pathway and the occurrence of the tire-leachables in 35 different hessian surface waters.

Influent and effluent of two conventional wastewater treatment plants were sampled over five consecutive days. 35 grab samples of hessian surface waters were collected to estimate the distribution of tire leachables, of these samples were enriched using solid phase extraction and analysed using a reversed phase liquid chromatography QTOF-MS system and screened for the prioritized leachables.

Based on the median ratio they were sorted into the categories 'elimination', 'decrease', 'consistency', 'increase' and 'formation'. Of the 28 detected tire wear leachables, eleven were classified as eliminated of which six were even below LOD in the effluent samples. DPG and dibenzylamine fell into the category "decrease". More than a third of the substances were classified as consistent, meaning that WWTPs act as distribution pathway for those tire wear leachables. Five substances showed an intensity increase passing through the WWTP. Thus, these substances seem to be transformation products of other substances present in the WWTP influent. The results of the surface waters showed similar patterns of the WWTP effluent samples. The four substances with a FOD > 60 % in the surface water samples were either among those with the highest intensity in the effluent (DPG) and/or they were classified as consistent (HMMM, benzothiazole-2-sulfonic acid and N-hexyl-N'-phenylfumaramide). This environmental screening demonstrated that previously unknown tire wear leachables can enter the water cycle through WWTP and are detectable in surface waters. However, an assessment of their environmental fate and associated risk is still not possible as there is a lack of data on source, occurrence and emission dynamics.

### **3.23.B Tire-Road Wear Particles: Analytical Possibilities, Challenges, and Current Knowledge of Environmental Impact**

#### **3.23.B.T-01 Tire and road wear particles contamination in infiltration ponds sediments: occurrence, spatial variability, size distribution and correlation with metals**

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Efficient stormwater management has become a major challenge. Sustainable urban drainage systems (SUDS) such as infiltration ponds have been implemented in sensitive areas, particularly in urban ones. These SUDS play a critical role in regulating water flow but also reducing pollutant load to downstream environment through decantation, soil filtration, photooxidation and biodegradation. In fact, runoff collected from road surfaces contains several categories of pollutants, including heavy metals, organic micropollutants, and microplastics such as tire and road wear particles (TRWP).

To improve our knowledge on the TRWP levels in runoff discharges and the capacity of SUDS to reduce TRWP load, we investigated the occurrence, spatial distribution and size distribution of TRWP in the sediment of an infiltration pond of the ring road in Nantes city (France). The sediment was fractionated between <36 µm and 500 µm, and analyzed by pyrolysis coupled with gas chromatography-mass spectrometry. Analyzes targeted two polymeric content of tires, namely, styrene-butadiene-rubber (SBR) and butadiene-rubber (BR), with the use of an internal deuterated SBR standard for calibration.

Measurements highlighted an SBR+BR gradient in the infiltration pond with contents ranging from  $1.28 \pm 0.05$  mg/g to  $7.15 \pm 0.29$  mg/g. Three distinct sections were delineated: entrance, middle and overflow. The sediment closest to the overflow was the most enriched in SBR+BR, consistent with the water runoff in the basin and the topography. Upon a closer examination of the size fractionation of SBR+BR content within the sediment from entrance, middle and overflow, the 63-160 µm fraction prevails. Furthermore, positive correlations between SBR+BR levels and particulate organic matter (POM) or metals (As, B, Cd, Cu, Li, Mo, Ni, Zn) ( $r > 0.76$ ,  $p < 0.05$ ) were observed. This suggests that TRWP may act as vector for metallic pollution and a carrier for POM, or behave in a similar way. Using a median SBR+BR-to-TRWP conversion factor of 13.2, the TRWP stock in the pond was determined and compared to the estimated cumulative TRWP input from road traffic. The calculation revealed similar amounts (of the order of 1.0 to 1.1 tons), suggesting that infiltration ponds can be an effective solution to limit the dispersion of TRWP into the environment.

#### **3.23.B.T-02 Bioaccessibility of Antioxidants, Antiozonants and Other Tire Hazardous Chemicals.**

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Tires, apart from being formed by rubber and filling materials (carbon and silica, principally), are made up of organic compounds which make the tire resistant and durable. The widely used of recycled tire crumb rubber (RTPCR), mainly sub-product of shredding process of end-of-life tires, could provoke a high exposure to human beings due to its presence on synthetic football fields and kid's playgrounds. In September 2023, the European Commission banned the use of recycled crumb rubber on synthetic fields giving the manufacturers 8 years to retire the material.

This study intends to assess the oral bioaccessibility of antiozonants, cross-linking and vulcanizing agents present in RTPCR. With this purpose, the Unified Bioaccessibility Method was selected to simulate the ingestion of the material. Real RTPCR

samples from sports and recreational facilities were put in contact with the four biological simulated fluids including saliva, gastric and duodenal juice and bile, attempting to simulate human digestion.

Afterwards, the organic compounds present in the fluids were extracted by Solid Phase Extraction (SPE) technique. The SPE process was optimized to obtain the best extraction conditions. Besides, ultrasound assisted extraction was performed to evaluate the total concentration of the compounds in the rubber matrix. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was the selected technique to identify and quantify the target compounds.

The results showed the bioaccessibility of all analytes studied. The bioaccessibility ranged from 0.01 % up to a high percentage depending on the compound. 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine) and its transformation product 6PPDq (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone) showed a moderate bioaccessibility when comparing with the other compounds. Benzothiazole (BTZ) showed the highest oral bioaccessibility. This work demonstrated for the first time the human bioaccessibility of these chemical agents.

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### **3.23.B.T-03 Effects of Weathered Tire Wear Micro- and Nanoparticles in the Model Estuarine Species Fish *Menidia Beryllina* and Mysid Shrimp *Americamysis bahia***

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The increase in plastic production worldwide and the mismanagement of plastic waste are leading the oceans to become a big waste bin. Million tonnes of plastic enter our ocean every year and car tires are one of the biggest sources. In the US almost 1,524,740 metric tons of tire wear particles (TWP) are released each year into the environment, deriving from tires undergoing friction on the road. For our experiments, we used a cryo-milled tire tread (CMTT) composite provided by the U.S. Tire Manufacturers Association. The composite was a representative mixture of what may be in our environment and contained three types of tire in proportions that are estimated for the U.S. (41% Passenger Car Tire, 14% Light Truck Tire, 45% Truck/Bus Tire). To better resemble what is happening in the real environment, leachate and micro- and nanoparticles were weathered through a solar simulator for 72h. Considering the limited available data regarding toxicity of environmentally relevant tire particles on organisms, this study aims to provide information about TWP exposure impacts on larval growth, internalization, behavior and intracellular reactive oxygen species (ROS) level in the model species Fish *Menidia Beryllina* and Mysid Shrimp *Americamysis bahia*. Particularly, 5 day post fertilization *M. Beryllina* embryos and 7-day old *A. bahia* were exposed to weathered and not weathered TP leachate and micro (1-20  $\mu\text{m}$ ) and nano (< 1  $\mu\text{m}$ ) tire particles at four concentrations (10, 100, 1000 and 10,000 particles/ml). At the end of the exposures, behavioral assays were performed using a DanioVision Observation Chamber, where each fish was subjected to a dark: light cycle stimuli. Growth measurements were assessed through the index ( $W = \text{width}$ ,  $L = \text{standard length}$ ,  $d = \text{days the organisms were exposed to tire particles}$ ) and organisms were cleared with CUBIC<sup>TM</sup> clearing reagents (Cubic-L and Cubic-R solutions) to visualize and count the internalized particles. ROS level was measured using a total reactive oxygen species assay kit 520 nm. Previous studies have already demonstrated tire particles to have behavioral toxicity and negative effects on growth and ROS level in our study species. This study has the potential to lead to a more comprehensive perspective of what organisms are experiencing in the real environment, reporting for the first time the toxicity due to environmentally relevant tire particles in the two model estuarine species *Menidia Beryllina* and *Americamysis bahia*.

### **3.23.B.T-04 Cocktail Chronicles: Investigating the Impact of Tire Particle Leachates Across Diverse Biological Models**

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Tire wear particles (TWP) significantly contribute to microplastic pollution. Despite extensive research on conventional microplastics, the environmental impacts of TWP and their leachates are poorly understood. This study investigates the cocktail effects of TWP leachate on diverse biological models using molecular, cellular, and organismal endpoints. Acute toxicity of TWP leachates (particle size <250  $\mu\text{m}$ ) was assessed via growth inhibition tests with marine microalgae (*Rhodomonas salina*), zebrafish (*Danio rerio*) embryotoxicity and behavioral tests, and in vitro assays for intracellular effects and hormonal response using reporter gene assays. Leachates were extracted in artificial seawater, followed by solid-phase extraction, and chemically characterized using spectrometric techniques. Exposure to TWP leachates (1.5 to 1000 mg/L) inhibited algae growth and induced zebrafish embryotoxicity, pigment alterations, and behavioral changes. Cell painting revealed pro-apoptotic changes, including cell rounding, mitochondrial toxicity, endoplasmic reticulum stress, and cytoskeleton disruption. The leachate exhibited potential endocrine disruption, mainly through antiandrogenic effects. Detected



organic toxicants include polyaromatic hydrocarbons and 4-tert-octylphenol. The present results indicate water-leachable organic compounds as the main causative agents of acute toxicity, contrary to other studies implicating mainly heavy metals. These findings underscore the multiple acute toxic effects of TWP pollution at various biological levels. Urgent measures are warranted to reduce such pollution in aquatic systems and enhance regulations on highly toxic tire additives.

### **3.23.P Tire-Road Wear Particles: Analytical Possibilities, Challenges, and Current Knowledge of Environmental Impact**

#### **3.23.P-Th400 Exploring the Potential of Proton-Transfer-Reaction Mass Spectrometry for the Analysis of Tire Wear Particles in Air**

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Tire wear particles (TWPs) contribute to the atmospheric particle burden in urban environments, and the increasing use of electric and hybrid vehicles is expected to further increase emissions of TWPs to the atmosphere. The qualitative and especially quantitative analysis of TWPs in air does however present distinct challenges. This is primarily due to the low atmospheric concentrations and complex chemical nature of TWPs, which contain polymers, organic additives and inorganic components (e.g., black carbon and zinc oxide) in variable amounts. Various analytical approaches are currently employed for TWP analysis, with Fourier-transform infrared (FTIR) spectroscopy and pyrolysis/thermodesorption gas chromatography-mass spectrometry (GC-MS) being most widely used. A less explored yet potentially promising technique for the qualitative and quantitative analysis of TWPs is proton-transfer-reaction mass spectrometry (PTR-MS). In our study, we coupled a PTR-MS analyzer to a custom-built pyrolysis unit, whose temperature was ramped from 250 to 700 °C at a rate of 8 °C/min. In our initial studies, we investigated the volatile pyrolysis products generated from pure tire polymers (polybutadiene, polyisoprene, polystyrene polybutadiene), polymer mixtures and real TWP samples. Each polymer generated a distinct mass spectral fingerprint, allowing to clearly differentiate the three polymers in their pure state, in polymer mixtures and in real-world samples. Furthermore, specific markers for each polymer were identified, providing a foundation for the quantitative analysis of TWPs. Real-world filter samples are currently being collected in urban air to investigate if the mass spectral signatures of TWPs can be detected in real-world samples.

#### **3.23.P-Th401 Microplastics and tyre wear particles infiltration in the soil of a roadside biofiltration swale**

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Urban roads stand as a major aspect of human activity. Understanding their contribution to microplastic pollution through macrolitter fragmentation and the generation of tyre and road wear particles is crucial to better assess the sources and fate of microplastics in terrestrial environments. In particular, the microplastic infiltration into roadside soils and soils of sustainable urban drainage systems via urban runoff remains to be assessed.

This study focuses on microplastic infiltration in the soil of a filtration swale alongside a high-traffic highway in the north of Paris, France. Ten soil cores were collected manually at a depth of 35 cm and were each subdivided by depth into four samples ([0-5], [5-15], [15-25], [25-35] cm). Each sample represented a mass of 10 g, that was separated into 2 subsamples for separate quantification of microplastics and tyre wear particles.

After specific treatment processes, the microplastic content of the samples was characterized and quantified using an automated micro-FTIR imaging analysis. The tyre wear particles content of the samples was indirectly assessed by quantifying the styrene-butadiene rubber (SBR) and butadiene rubber (BR) content of the samples by Pyr-GC/MS analysis.

Results revealed a median concentration of 5.4 µg MP g<sup>-1</sup>, for a median abundance of 4.0 MP g<sup>-1</sup>. Surface samples presented a significantly higher microplastic abundance than deeper layers, with a median abundance of 47 MP g<sup>-1</sup> against 1.8 MP g<sup>-1</sup>. SBR concentrations were significantly higher than microplastics for all samples, with a median concentration of 150 µg SBR g<sup>-1</sup>, suggesting a much higher tyre wear particles concentration in the roadside soils. Interestingly, SBR concentrations presented a similar vertical profile as microplastics, with a decrease in concentration in the deeper samples.

These findings advocate for an increased attention to tyre wear particles in microplastic studies, particularly within urban contexts, as they likely represent high concentrations and follow similar transport pathways as other microplastics.

#### **3.23.P-Th402 Exposure to Cryogenically Milled Tire Tread Contamination May Induce Functional Changes in Periphyton Communities**

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Periphyton communities are complex matrixes composed by prokaryotic and eukaryotic organisms embedded in extracellular polymeric substances (EPS) growing on sediments and rocks of freshwater streams. These autotrophic communities are at the base of the aquatic food webs, being involved in crucial ecosystem processes. Changes at this level can have far-reaching effects on the ecosystem. This is the more important as periphyton covering large surfaces of the stream bottom, may act as a sink for a diverse range of pollutants, including tire and road wear particles (TRWP). TRWP are produced due to abrasion, being deposited on the roadside and, consequently, transferred to the surrounding soil and surface waters. Once in the aquatic environment, the potential interactions of TRWP, and respective leachates, with periphyton communities are unknown. In this study, we aimed at exploring the effects of tire wear particles on periphyton, using cryogenically milled tire tread (CMTT) as currently available surrogate to simulate TRWP. Periphyton grown in flow through channels using stream water (14 d), was subsequently transferred to microcosms and exposed to 0, 50, 500 and 1000 mg CMTT/L (+14 d), with medium exchange every 3-4 d. We measured photosynthetic activity and chlorophyll *a* content as functional endpoints, as well as, community structure and composition assessed via flow cytometry (FC) and next generation sequencing (NGS). Additionally, we recovered the CMTT particles, from which metals and organic chemical content of both medium and periphyton matrix were measured. We hypothesized that CMTT particles would accumulate in the matrix overlaying periphyton, along with a potential bioaccumulation of metals and organic chemical resulting from the particles' leaching. CMTT was accumulated on the biofilm surface; however, we could only measure it at 500 and 1000 mg/L, as for lower concentrations, 0 and 50 mg/L were below the limit of detection for weight. Moreover, the photosynthetic efficiency decreased by 10 % at 1000 mg/L. This decrease in photosynthetic activity was supported by the decrease in chlorophyll *a* content at 500 and 1000 mg/L. Further analysis are being conducted on the community structure and composition. Follow-up experiments will analyse whether the measured effects result from chemical exposure or shading effect, or both.

### 3.23.P-Th403 Presence of 6PPD, 6PPDq and Related Compounds in Real Water Samples Contaminated by Coming Into Contact with Tire Rubber Surfaces

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Tire rubber contained many compounds, such as antiozonants, vulcanizing or crosslinking agents. 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine) is an antiozonant and its degradation product, 6PPDq (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone), is linked to the mortality of aquatic species. These chemical agents are introduced into the environment through tire wear particles or through crumb rubber, obtained from recycled tires, used as infill in artificial turf fields or as playground flooring. Water leachates from these types of surfaces contribute as well to the introduction of these substances into aquatic ecosystems, as well. Therefore, the identification and quantification of these organic compounds in water matrixes is crucial.

In this study, an analytical methodology for the simultaneous detection of several tire rubber compounds is proposed. The methodology is based on a miniaturized sample preparation technique, Solid Phase MicroExtraction (SPME), for which no solvent extraction is required, and no hazardous chemicals are employed. The methodology was applied to real water samples in contact with rubber materials, some of them collected from playgrounds, football fields, and parking places. Some of the target analytes, including 6PPD and 6PPDq, were found in the real waters demonstrating their leaching from the rubber.

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### 3.23.P-Th404 Biodegradation of tire-related phenylenediamines and their transformation products identification

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Tire-related chemicals are receiving considerable attention as potential contaminants in the environment. N-(1,3-dimethylbutyl)-N'-phenyl-1,4-benzenediamine quinone (6-PPDQ) is a transformation product (TP) of the tire additive 6-PPD and has been reported to contribute to mortality of some Salmon in the US. Although a number of TPs have been identified from tire additives under environmental conditions such as oxidation and photolysis, there has been limited research on their biodegradation products, which may be of greater environmental relevance. In this study, the biodegradation of the most prominent PPDs and their TPs, N,N'-diphenyl-p-phenylenediamine (DPPD), N-isopropyl-N'-phenyl-1,4-phenylenediamine (IPPD), 6-PPDQ, IPPDQ, 4-nitrosodiphenylamine (NO-DPA), and 4-hydroxydiphenylamine (4-HDPA) was studied. Their corresponding transformation products (TPs) were characterized using ultra-performance liquid chromatography coupled to high resolution mass spectrometry. A total of 41 biodegradation TPs were detected by the non-target screening and tentatively identified from 6-PPDQ, IPPD, NO-DPA, 4-HDPA, and DPPD. Of these, 17 were stable until 28 days. All studied chemicals exhibited complete primary degradation (removal of parent compound): It varied between a few hours (NO-DPA) to 9 days for 6-PPDQ, with 4-HDPA and DPPD showing removal times  $\leq 3$  days. Nine of the TPs of 6-PPDQ (6PPDQ\_342, 6PPDQ\_367, 6PPDQ\_315, 6PPDQ\_313, 6PPDQ\_263A and B, 6PPDQ\_319A and B, 6PPDQ\_317) showed an increasing trend until the 14<sup>th</sup>

day and then stabilized until the last day. One of the hydroxylation products of 6-PPDQ (6PPDQ\_315) has been reported to be formed in liver cells of rainbow trout. All detected TPs differ from those reported from abiotic ozonation studies. This suggests that other moieties of 6-PPDQ react with bacterial enzymes than with the electrophilic ozone. Also, one of the high intensity TP of HDPA, HDPA\_204 could be a coupled TP of the two HDPA molecules. Although self-coupled reactions are unlikely to occur in the environment, this suggests that in more complex environments, such addition reactions may occur with other reaction partners. The discovery and identification of biodegradation products of these tire-related chemicals can be used to further assess their occurrence in the environment and supports risk assessment.

### **3.23.P-Th406 Characterization of the Impact of Standard Soils as Sample Matrix on the Pyrolysis Products of Polystyrene and Tire Particles**

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While Pyrolysis coupled to gas-chromatography mass spectrometry (Py-GC-MS) is widely applied in the field of tire and road wear particle (TRWP) analysis, major limitations remain. Apart from the fact that tire compositions vary significantly between manufacturers and that there are no reference materials, the applied Py-GC-MS methodologies are not harmonized and oftentimes validation data is lacking. This makes it difficult to compare the results. Validation is also important given the uncertain effects of the matrix on pyrolysis but also regarding the generation of marker substances from the matrix. To limit its impact on the analysis additional, validated sample preparation steps are advisable.

Aim of this work is to investigate the effects of three standard soils (sand, sandy loam, clayey loam) on the pyrolysis of polystyrene and a tire particle mix, with and without sample preparation. Here, potassium hydroxide, described as a promising reagent for sample digestion in literature, will be tested. Validation parameters will be determined to investigate, whether the matrix effects can be sufficiently accounted for by the internal standard calibrated Py-GC-MS method.

Measurements are performed on a Curie-point pyrolysis unit coupled to a GC-HRMS. The commercially acquired standard soils are spiked with a polystyrene standard or tire particle mix at three concentration levels. Samples are spiked with deuterated polystyrene and one half subjected to digestion with KOH at 60°C for 24 h as sample preparation. Afterwards fivefold measurements of the samples are performed to enable validation including recovery, as well as intra- and interday repeatability.

The experiment shows the effect of sample matrix on pyrolysis. It reveals whether the Py-GC-MS approach with internal standard calibration used in many papers is able to account for matrix effects caused by different soil types. Furthermore, the suitability of sample digestion with KOH will be assessed. The results of this work will provide insight into the pyrolysis process and matrix effects and help to further the understanding of Py-GC-MS as tool for the analysis of TRWP.

### **3.23.P-Th407 Trace Metal Adsorption on Tire and Road Wear Particles in Surface Waters – A Problem for Water Quality?**

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German-wide up to 20,000 t/a of tire wear particles (TWP) are emitted into the aquatic environment. Regarding the total amount of microplastic emissions, TWP contribute to about 30%. Since there is an increasing number of motorised vehicles, a decreasing of TWP emissions is not expected. Recent researches mainly focus on the characterization and on the ecotoxicological effects of TWP on aquatic organisms. Thereby the focus lies on the particles themselves and possible leachates but not as a carrier for adsorbed pollutants.

The overly hydrophobic surface of TWP is capable in adsorbing substances like trace elements. The so bonded pollutants can be ingested by organisms and freed up inside their body. So those pollutants may become bioavailable.

Our investigations tried to characterize these adsorptive effects based on environment-related samples. Therefore, we used different kinds of tire wear particles samples with various estimated tire wear particle content. These samples were either produced by filing, collected behind a driving car (industry partner) or by sampling motorway tunnels (Elbtunnel, Hamburg, Germany). This material, so called *tire and road wear particles including road sediment* (TRWP+RS), contains tire wear particles encrusted with road wear and road related particles as well as other fine particles on the road. Water samples were taken from the Freiburger Mulde, Central East Germany, as an example of a river containing a high amount of trace elements and heavy metals.

In the laboratory TRWP+RS samples were weighed into glassware and filtered water samples (0.2 µm) were added to obtain an environmentally relevant concentration of TRWP+RS. After shaking for 24 h the samples were filtered (0.2 µm) and the element content of the solid samples determined by digestion in a microwave and ICP-MS/MS.

Assessing the adsorption results on the particles with the suspended matter classification of LAWA, we could show that the TRWP+RS itself does not display an endangering except for zinc and copper. Compared to the amount already present in the

rivers Freiberger Mulde and Elbe, one could argue not to have an endangering at all. However, when it comes to the adsorption of heavy metals and arsenic an endangering due to chromium, nickel, copper, zinc, and cadmium can be seen due to deterioration of the chemical quality class.

### **3.23.P-Th408 Comprehensive Approach to National Tire Wear Emissions**

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The use of vehicle tires has been identified as a major source of microplastics in the environment and an increasing source of urban particulate air pollution. In light of increasing traffic volumes, increasingly heavier and more powerful vehicles due to trends and electrification, and the lack of regulation of tire wear, methods to estimate and follow changes in national emissions are needed as input for environmental effect assessments. Emission estimations of tire wear are made either based on the mileage approach or the sales approach. Both approaches have drawbacks related to the availability and representativity of data needed. This study aims to investigate if and how the mileage approach can be improved by using emission factors for passenger cars and light-duty vehicles based on own measurements and emission factors from literature for heavy-duty vehicles and buses. An approach with emission factor adjustments based on weight and number of tires, in combination with highly detailed mileage data, has been evaluated. Sales approach calculations have been used to validate the method. A secondary aim has been to use the new mileage approach framework to calculate the national tire wear emissions for Sweden. These calculations resulted in slightly lower total emissions than previous estimations, with higher emissions for passenger cars and light-duty vehicles, but lower emissions for heavy-duty vehicles and motorcycles. Passenger cars constitute more than half of the total emissions. It is concluded that even though the framework offers greater detail, giving increased possibilities to adjust for changes in emission factors and mileages in specific vehicle categories, the challenges posed by, e.g., lack of measured emission factors for heavy-duty vehicles and uncertainty in the quality of mileage statistics makes the estimations uncertain. Important future research suggestions are establishing reliable emission factors, especially for heavy-duty vehicles, and initiating research to better understand how climate, road network, surface properties, and vehicle fleet characteristics affect emission factors.

### **3.23.P-Th409 The Identification and Quantification of Tire and Road Wear Particles in Osaka Bay, Japan, by Two Analytical Methods**

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Tire and road wear particles (TRWP) are formed at the frictional interface between tires and the road surface. The mixture of tire tread and road pavement material can migrate from the road surface into receiving waters during precipitation events. Based on the density of TRWP, they are expected to partition into sediment. The lack of mass-based measurements in marine environments represents an uncertainty in environmental risk assessments and fate and transport models. This study investigated the abundance and distribution of TRWP in Osaka Bay, Japan. Surface water and sediment grab samples were collected from nine locations within the bay in June 2023. Sediment traps were deployed for a period of approximately nine weeks at three locations along a 13.5 km transect from the mouth of the Yodo River. Surface water samples, collected in large volumes, underwent filtration through 100, 10, and 0.5 µm bag filters using a water pumping system to capture retained solid particles. After drying, large particles (>5 mm) were removed, and the solid samples were homogenized and sub-sampled. Sediment and retained solid samples were analyzed for TRWP using a microfurnace Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) method (ISO Technical Specification 21396:2017, modified) and a particulate zinc method. In addition to TRWP analyses, sediment samples were analyzed for total organic carbon and grain size distribution. Surface water grab samples were collected and analyzed for total and dissolved organic matter as well as total suspended solids. TRWP results will be compared between the two analytical methods to assess the consistency in detecting and quantifying TRWP in marine samples. The concentrations of TRWP in Osaka Bay will be compared to previously reported findings in Lake Biwa and the Yodo River, contributing to an understanding of spatial and temporal variations of TRWP in this heavily populated and industrialized watershed. The results of this study will enhance our knowledge of TRWP concentrations in a marine environment and inform efforts to assess potential environmental risks, calibrate mass-balance models, and develop strategies for mitigating TRWP in the environment.

### **3.23.P-Th410 The measurement of tire and road wear particles in road surface, roadside soil and retention basin samples in the Chesapeake Bay watershed, USA**

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When vehicles travel on a road, the tires and road surface interact with each other, resulting in the generation of tire and road wear particles (TRWP). The mixture of tire tread and road pavement material can migrate from the road surface to soils adjacent to the road via wind dispersion and into receiving waters during precipitation events. A comprehensive study of TRWP was conducted from June to September, 2023, across various environmental matrices within the Chesapeake Bay watershed, USA. Sampling efforts included road surfaces, roadside soil, stormwater, surface water and sediment in the Baltimore, Maryland, area. Road surface samples were collected using a high-efficiency vacuum from a tunnel, a high-use road and a low-use road. Surface soil samples were collected from 1 to 30 meters from a high-use road in a series of three transects. Stormwater, surface water and sediment samples were collected from a retention basin receiving runoff from the high-use road.

Stormwater and surface water samples, collected in large volumes, underwent filtration through 100, 10, and 0.5 µm bag filters using a water pumping system to capture retained solid particles. Solids were screened through a 5 mm sieve to remove large particles and homogenized in a ball mill. TRWP concentrations were analyzed using a microfurnace Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) method (ISO Technical Specification 21396:2017, modified) and a particulate zinc method. Soil and sediment samples were analyzed for total organic carbon and grain size distribution. Surface water grab samples were collected and analyzed for total and dissolved organic matter, as well as total suspended solids. Comparison of results obtained from these two methods will provide valuable insights into the consistency between these analytical approaches in detecting and quantifying TRWP in a variety of environmental matrices. Additionally, TRWP concentrations from this study will be compared to previously reported findings in the Chesapeake Bay watershed, contributing to our understanding of spatial and temporal variations of TRWP in aquatic systems. The outcomes of this study will advance our knowledge of TRWP, aiding in estimating emission factors, understanding TRWP migration via wind dispersion and stormwater runoff, calibrating mass-balance models, assessing environmental risks and developing strategies to mitigate TRWP in the environment.

### **3.23.P-Th411 Exploring the Presence and Spatial/Temporal Patterns of Tire and Road Wear Particles in the Seine River, France**

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Tire and road wear particles (TRWP), formed at the frictional interface between tires and the road surface, are widely present in the environment. Tire tread and road pavement materials are denser than water but can be washed from the road surface into receiving water bodies, ultimately depositing into sediment. TRWP have been identified in various environmental compartments but limited research has been conducted on estuarine samples. The main objectives of this study were (1) to determine TRWP in surface water and sediment along the Seine River by two different methods: refined Pyrolysis Gas Chromatography/Mass Spectrometry (Py-GC/MS) and particulate zinc, (2) compare the results from the present study with past TRWP data from the Seine River, and (3) understand the sources and fate and transport processes of TRWP. Surface water and sediment samples were collected from eight locations along the Seine River, France. These locations included upstream of Paris, within the Paris metropolitan area, downstream of Paris near smaller urban areas, adjacent to an undeveloped area and near the confluence of the river and the English Channel. At each location, surface water was collected for: the analysis of matrix characterization parameters (i.e., total organic carbon (TOC), dissolved organic carbon, and total suspended solids), retained solids for the analysis of styrene-butadiene/butadiene rubber, natural rubber, and total TRWP, and sediment samples for TOC, grain size, and TRWP analysis. The Py-GC/MS analysis of TRWP in surface water ranged from 0.54 to 10 µg/L and from <180 to 2,300 mg/kg in sediment. Surface water samples showed higher concentrations near Paris - consistent with the increased traffic density and more impermeable surfaces. The highest sediment concentration was observed downstream of a wastewater treatment plant - suggesting a potential source to the environment. TRWP concentrations estimated by the zinc method (1.8 to 5,120 mg/kg) were on average more than three times higher than reported by the refined Py-GC/MS method. Comparisons of the study's results of total Zn with available historical data revealed a decrease in TRWP over the last decade, suggesting an improvement in overall water quality in the region. This study is the first to report TRWP concentrations using two different methods in estuarine samples.

### **3.23.P-Th412 Tyre Wear and other Microplastics in Snow in Urban Traffic Environments**

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Microplastics from road traffic, mainly from tyre wear, are globally considered to be one of the largest sources of microplastic contamination in the environment. Plastics can either be deposited in the road vicinity, at the roadside and in ditches or spread via stormwater and air to the environment and receiving water bodies. In cold climates, microplastics, as well as other traffic-related pollutants, can be temporarily stored in snow and ice on and around roads and streets. The location and concentration of these pollutants is influenced by winter operations, where ploughing and skid control contribute to redistribution, and by melting and compaction of ice and snow. This creates reservoirs of microplastics and other pollutants, which are released into stormwater or surrounding soil during thaws, but also provides an opportunity to reduce the spread of microplastics by managing snow and ice appropriately. In this work, a case study of microplastics in snow has been carried out in the municipality of Karlstad in Sweden, to get an idea of the potential variation, both in terms of concentration and total amounts in relation to traffic and the location of the sampling in the street environment. Microplastics have been analysed by pyrolysis GC/MS to identify tyre-specific polymers in combination with eight commonly occurring plastic types. In addition, six municipalities in different parts of the country responded to a questionnaire on microplastics in snow and urban snowmelt management. The results show that microplastics related to tyre wear (rubber polymers) tend to be present in higher concentrations on and near the carriageway. Other plastics show a less clear link to traffic. Along a salted bicycle lane, an elevated level of polypropylene, from which the brush of the sweep-salting machine is made, could be detected in the surface layer of the snow. In general, knowledge about microplastics in urban snow is low in the municipalities that responded to the survey. The results imply a potential to differentiate handling of different urban traffic snow compartments to mitigate microplastic emissions via runoff from snow melting.

### 3.23.P-Th413 Tyre Wear Particles at a Swedish Highway– Occurrence in Stormwater, Sediments and Snow

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The presence of tyre wear particles (TWP) in stormwater runoff and sediments has been previously confirmed and quantified, increasing concerns due to their potential impact on the environment and human health. Understanding tyre wear occurrence, spread, and transport in stormwater can aid prediction of TWP concentrations and prioritisation of remediation measures. The aim is to present a synthesis of a large research investigation of TWP in water, sediments and snow samples at the highway E18 in Sweden. The goals of the investigation were to: i) quantify TWP occurrence in a highway stormwater system, ii) assess TWP transport in the stormwater system during rain, and iii) quantify TWP in snow at different distances from the road. A comprehensive campaign for collecting environmental samples has been conducted at Testsite E18, located by the Swedish highway E18, which is a road research facility belonging to the Swedish Transportation Administration. The facility offers the possibility to sample water and sediments in a stormwater system connected to the highway (gully pots, wells, ditches and receiving water), sample water from direct runoff and sample snow along the highway. Water samples were analysed at size fraction 1.6–500 µm, and snow and sediment samples were analysed at size fraction <500 µm. Part of the samples were also analysed at size fraction 1.6–20 µm. The samples were analysed for styrene butadiene rubber and butadiene rubber using Pyrolysis GC-MS, and the tyre wear mass was calculated using Monte Carlo simulations based on traffic data, as proposed by Rødland et al. (2022) [1]. The median TWP <500 µm concentrations detected in the water grab samples ranged between 0.4 and 17 mg/l. The sediment samples contained median TWP <500 µm concentrations ranging from below detection limit up to 25 mg/g dry weight. In addition to these results, concentrations of TWP in stormwater samples from rain events and snow samples will be presented. This study is one of the first to investigate TWP concentrations in different parts of a stormwater system, the variation of TWP concentration during a rain event, and the occurrence of TWP in snow alongside a highway.

[1] R Rødland ES, Samanipour S, Rauert C, Okoffo ED, Reid MJ, Heier LS, Lind OC, Thomas KV, Meland S. 2022. A Novel Method for the Quantification of Tire and Polymer-modified Bitumen Particles in Environmental Samples by Pyrolysis Gas Chromatography Mass Spectroscopy. *J Hazard Mater* 423 Part A: 127092.

### 3.23.P-Th414 Detection and Effects of pneumatic microparticles in aquatic environments – Case study in run-off water

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Microparticles resulting from tire wear on roads are now recognized as a significant contributor to microplastic pollution. These micrometric residues mainly come from tire tread, which contains chemicals such as plasticizers, antioxidants, reinforcing agents, and fillers. Various studies have demonstrated the toxic potential of these compounds for aquatic organisms. Therefore, studying the kinetics of releasing these particles into the environment and their potential toxicity is essential. The PLASTyre project aims to detect and quantify tire microparticles in runoff water via stormwater retention structures in close collaboration with local stakeholders (Nouvelle-Aquitaine region). To this end, a methodological development in µFTIR verified by GC-MS pyrolysis will be carried out. The project's second objective is to assess the potential toxicity of tire micro-particles using two types of particles: Cryogenically Milled Tire Tread and Tire and Road Wear Particles (TRWP). Macrofragments of tires will also be artificially aged in a UV chamber and then agitated to obtain leachates. Organisms' exposure to these leachates will allow us to assess the effect of the harmful compounds potentially released. Toxicity tests will be performed on various study models, including marine bacteria, bivalves, fish, and amoebae, via direct exposure to particles or leaching. Thanks to a combination of detection techniques and methodological development using state-of-the-art equipment, we could potentially identify the presence of TRWP in areas with dense traffic, as recent studies on the subject suggested. Some articles have revealed some toxic effects of tire microparticles on specific study models. It will, therefore, be interesting to study various other biological models belonging to different taxa using concentrations representative of the environment and lethal and sub-lethal endpoints. This project could provide new insights into the current analytical capabilities, fate, and potential toxicity of these compounds in the environment.

### 3.23.P-Th415 Tyre and Road Wear Particles and Additive Chemicals in Roadside Sustainable Drainage Systems (SUDS)

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Tyre and Road Wear Particles (TRWPs) are an understudied source of microplastic (MP) pollution in the aquatic environment. Tyres also leach toxic chemical additives. There are several routes from roads to the environment, including surface runoff. One method of road drainage is by Sustainable Drainage Systems (SUDS) such as retention ponds, which retain runoff for a period before releasing into the wider environment, allowing for sedimentation of particles and removal of contaminants via chemical and biological processes. However, there is a current lack of sampling methodologies for adequate and representative sampling of TRWPs and additive chemicals simultaneously.

A portable stainless-steel sampler was designed for simultaneous sampling for additives and particles over 24 hours utilizing a battery powered peristaltic pump. Stainless-steel sieves (250, 100, and 50 µm) facilitated in-situ fractionation of TRWPs.

Developed analytical methods enabled the quantification of TRWPs by microscopy and analysis of 25 additives by UHPLC-MS/MS in road runoff.

Particles >50 µm were quantified and categorized in samples collected from a SUDS retention pond serving the Aberdeen Western Peripheral Route (AWPR) road in North-East Scotland. A total of 70.7 particles/L were found, 43.4 of which were black particles/TRWPs and 17.6 were white or reflective, likely originating from road paint. 86.5% of particles were within the 50-99 µm fraction, including 83.7% of suspected TRWPs and 97.3% of white/reflective particles. Seven chemical additives, (1H-benzotriazole, 5-methylbenzotriazole, N,N-diphenylurea, 1-cyclohexyl-3-phenylurea, 2-aminobenzothiazole, 1,3-diphenylguanidine, and 2,4,6-tris(bis(methoxymethyl)amino-1,3,5-triazine) were detected. Concentrations ranged from 0.0084±0.000050 - 0.18±0.012 µg/L and 0.073±0.0058 - 0.44±0.14 µg/L in inlet and outlet samples respectively. Interestingly, negative removals were observed for all seven additives between -28 and -774 % suggesting leaching from TRWPs in retention ponds.

TRWPs and suspected paint fragments have been found in samples from SUDS ponds serving the AWPR along with seven additive chemicals. This suggests that road drainage could act as a pathway for plastic pollution into the wider environment. Future work will investigate the impact of weather conditions on the suitability of SUDS to remove TRWPs and additive chemicals and compare different drainage designs serving different road types and driving conditions.

### 3.23.P-Th416 Chemical Profile and Toxicity of Leachates from Different Types of Tires

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Studies have demonstrated that tires contain a wide range of compounds in tires and tire leachates, including synthetic and natural rubbers and natural rubber, metals and organic compounds. To evaluate which types of tires may have the highest impact on the environment based on the chemical profiles and the toxicity of their leachates. Our main aim was to investigate the concentration levels of rubbers, selected metals (zinc, nickel and copper) and selected organic compounds (6-PPD-q (N-(1,3-dimethylbutyl)-N'-phenyl-pphenylenediamine, 6-PPD), HMMM (hexa(methoxymethyl)melamine) and DPG (N,N'-Diphenylguanidine) in leachates from 4 tires (winter studded, winter non-studded, summer, truck) (35mg/L) across 21 days of leaching. We tested the toxicity of the leachates in the freshwater microalgae *Raphidocelis subcapitata* and zebrafish embryos *Danio rerio*, to determine if the chemical composition of the four tires had different effects on these organisms.

The synthetic rubber content was highest in the truck tire (43%). The highest metal levels were found for zinc (4.8-202 µg/L), and levels increased over the 21 days (ANOVA, p<0.0001). A significant difference between tire types were found (p<0.05), with the highest levels coming from the truck tire (111 ± 73 µg/L). All organic compounds increased in the leachates across the 21 days, except for 6PPD and HMMM. The 6PPD-quinone levels increased from 610 to 4230 ng/L across 21 days (p<0.0001). No significant difference in 6PPD-q levels were found between the tire brands (p=0.509) or the tire types (p=0.138). 71% of the variation (RDA, p<0.0001) could be explained by the brand, vehicle type, seasonal tire type and time of leaching. The truck tire was the most toxic to *R. subcapitata* (EC50=28.44 mg/L. In terms of specific toxicity, ROS formation, metabolic activity and neutral lipid content were the most affected parameters. parameters. A small effect in survival and hatching rate were observed for zebrafish embryos, with truck tire showing the highest effects.

The different responses in microalgae and zebrafish suggest a correlation with the chemical profile of the CMTT. Our results demonstrate the importance of assessing the chemical profiles and toxicity of individual tires, as well as in mixtures. This is particularly important to understand the impact from tires and their leachates in different environmental compartments.

### 3.23.P-Th417 Investigating toxicity pathways of crumb rubber-derived particles and leachates on blue mussels *Mytilus edulis*

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Tyre wear particles together with end-of-life tyres (ELTs) are major contributors to microplastic pollution in aquatic ecosystems. Moreover, increasing concerns revolve around their complex chemical composition. During manufacturing, additives as aromatic heterocyclic compounds, aromatic amines and heavy metals are added to the rubber polymer matrix to enhance its properties. Under environmental conditions, these chemicals can leach into the ecosystem, posing a significant risk to aquatic biota. However, studies with aquatic species have primarily focused on the effects of tyre particles alone and/or individual additives, highlighting a critical gap in the discrimination between their toxicity drivers and pathways. Thus, the main objective of this study was to assess and differentiate the impacts of car tyre particles, using crumb rubber from ELTs (TP, <300µm), car tyre leachates (L) and car tyre particles after 14 days of leaching (TPL, <300µm) on adult mussels *Mytilus edulis*. Mussels were chosen as model organisms due to their ecological and commercial roles. Mussels were exposed for 28 days to 0.1 g/L of TP, L and TPL, followed by 1 week depuration. Three ecotoxicological biomarkers, namely lysosomal membrane stability (LMS), acetylcholinesterase activity (AChE) and lipid peroxidation (LPO) were evaluated at five different

sampling points (0, 7, 14, 28 and 36 days) in different mussel tissues. Organisms were also analyzed for particle and chemical bioaccumulation. Preliminary data indicates treatment and time dependent responses. Lower LMS values were observed at day 7 for TP and day 28 for TPL, indicating changes in mussel health status. TP also led to an increase in LPO levels after 7 days of exposure, potentially indicating oxidative stress. AChE activity was significantly inhibited by L at day 7 and TP at day 28, suggesting a neurotoxic effect. Additionally, AChE levels for L and TP remained below the control values even after depuration, indicating continuous neurotoxicity. This study reveals insights into the mechanisms of toxicity associated with tyre-derived particles and their leachates towards marine biota. It further suggests a significant early role of the chemical component, as well as a synergic effect between additives and particles. Nonetheless, there is still a need for further investigation on the specific toxicity pathways of tyre particles and leachates in organisms to thoroughly assess their potential environmental implications.

### **3.23.P-Th418 Understanding the Impact of Chemical Leachates from Car Tire Rubber on Marine Microalgae: Insights into Toxic Mechanisms and Ecosystem Implications**

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Micronized car tire rubber (CTR) particles contribute significantly to the presence of plastic particles in the environment, raising concerns about the release of chemical additives into the marine ecosystem. Some of these additives are highly toxic to aquatic organisms, posing a serious threat to microalgae, at the basis of the aquatic food web. As keystone organisms, any disturbances in microalgae communities can have far-reaching consequences for the entire aquatic ecosystem. This study investigates the toxic effects of chemicals associated with CTR, released during 7 and 14 days of leaching, on four marine microalgal species: *Skeletonema pseudocostatum*, *Rhodomonas baltica*, *Isochrysis galbana*, and *Tetraselmis suecica*.

Screening tools such as flow cytometry and pulse amplitude modulated fluorometry were initially employed to assess general toxicity, focusing on parameters like growth rate, cell size, complexity, natural pigments content, and photosystem II performance. Results revealed that the 14-day leachate exhibited the highest toxicity across all species, with *S. pseudocostatum* displaying the greatest sensitivity ( $EC_{50}=3.26$  mg/mL). Subsequently, a high-throughput methodology was employed at sub-lethal levels to investigate the specific toxic mechanisms of CTR leachates. Analysed endpoints included metabolic activity, cell viability, cytoplasmic and mitochondrial membrane potentials, reactive oxygen species (ROS) formation, lipid peroxidation, neutral lipids, cellulose, and DNA contents.

The study uncovered the specific toxic mechanisms of CTR leachates on crucial components of microalgae cells, with notable impacts on ROS formation, oxidative stress, and a significant reduction in cellulose content. This comprehensive analysis provides new insights into the toxicity mechanisms of CTR leachates on marine microalgae, emphasizing the importance of understanding the potential impacts of plastic-associated chemicals on the marine ecosystem. This research was supported by the MicroLEACH project (#295174), funded by the Norwegian Research Council.

### **3.23.P-Th419 A Comparison of the Uptake of Tyre Particles via Suspension and Surface Deposit Feeding in the Estuarine Amphipod, *Corophium volutator***

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Tyre particles, produced from abrasion between car tyres and roads, have been reported as a major source of microplastic pollution for aquatic environments. The release of tyre particles has been recognised as an environmental pollutant since the 1970s, but only in the last decade has research begun to elucidate the extent of this contamination in the natural environment. In recent years, toxicological research has begun to focus on whether the ingestion of tyre particles can cause deleterious impacts in biota. In this study, I expose the estuarine amphipod *Corophium volutator* to environmentally relevant concentrations of tyre particles to quantify the adherence and ingestion of tyre particles via two different feeding modes: suspension feeding and surface deposit feeding. I hypothesise that *C. volutator* will ingest tyre particles, within their optimal prey size range, via both feeding modes. *C. volutator* were collected from a clean field site and acclimated for 14 days in clean sediment. Cohorts of three *C. volutator* were then placed into their respective exposure treatments dosed with environmentally relevant concentrations of tyre particles ( $0.1$  g L<sup>-1</sup>) under constant environmental conditions. For the suspension feeding treatment, *C. volutator* were placed in artificial burrows suspended within seawater, and for the surface deposit treatment, *C. volutator* were allowed to create burrows in natural sieved sediment. Following an hour exposure, the individuals were removed from the treatments and imaged for adhered tyre particles and digested using NaClO for the ingested particles. In the suspension treatment, tyre particles were found to be adhered and ingested by all individuals. There was a significant difference in where the tyres aggregated (Kruskal-Wallis,  $df=6$ ,  $p<0.001$ ). *C. volutator* had higher numbers of adhered particles to the antenna compared to other body parts, this may pose problems for the organism as the role of the antennae is to sense the environment and aid in crawling behaviour. If the antennae are saturated with tyre particles, it may lead to behavioural changes and being more susceptible to predation as their ability to sense their environment may be altered. The outcomes of this study will elucidate the risk tyre particles pose to biota in benthic estuarine and coastal environments.

### **3.23.P-Th420 It's Getting Confusing: 6PPD-quinone Induces Developmental Cardiotoxicity in Fathead Minnow (*Pimephales promelas*) Embryos Following Microinjection**



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N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), an oxidation product of the rubber tire antioxidant, 6PPD, is an emerging contaminant of concern that was first identified as the cause of Urban Runoff Mortality Syndrome, a mass lethality event observed in coho salmon (*Oncorhynchus kisutch*). Acute lethality of 6PPD-quinone is highly species-specific, with the only sensitive species identified to date being some salmonids. Using waterborne exposures, we previously found that neither sexually mature fathead minnows (*Pimephales promelas*) exposed to 6PPD-quinone at 9.4 µg/L, or embryos exposed to 6PPD-quinone at 40 µg/L suffered acute lethality. Further, embryos did not show any developmental malformations. Using metabolomics and transcriptomics, we showed evidence of sublethal oxidative stress in adults. Based on these findings, we selected fathead minnows as a model species to further investigate potential sublethal effects of 6PPD-quinone. To ensure consistent and precise dosing, newly fertilized embryos were microinjected with 6PPD-quinone at nominal doses of 18.5, 55.5, 166, 500, and 1500 ng/g of egg. Consistent with our previous findings, exposure to 6PPD-quinone did not induce mortality up to 12 days post-fertilization. However, a suite of developmental malformations was observed, including spinal curvature, pericardial edema, abnormal heart morphology, lack of common cardinal vein development, and hemorrhaging at sporadic anatomical areas. Frequency of these effects ranged from 30-100%, depending on dose. To understand the mechanisms of these results, whole transcriptome RNA sequencing and 6PPD-quinone metabolite analysis is currently being performed. These studies will provide novel insight into mechanisms of toxicity of 6PPD-quinone to fishes.

### **3.23.P-Th421 Chronic Toxicity of Tire Additive 6PPD and its Oxidized Substance 6PPD-Q in the Reproduction and Locomotion of *Daphnia magna***

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD; CASNR: 793-24-8), which is added to tires as an antioxidant and antidegradation agent, is known to have excellent anti-ozone properties that help prevent tire wear. While 6PPD has been used in tires since the mid-1960s, it has recently been found to convert to the oxidation product N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-Q; CASNR: 2754428-18-5) during use, and its toxicity to fish has been of particular concern. To evaluate the chronic toxic effects of 6PPD and 6PPD-Q on aquatic organisms, a 38-day toxicity test was conducted using the standard test species, the common water flea (*Daphnia magna*). On post-exposure day 21, the heart rate, body length and total moving distance of *D. magna* were measured. Additionally, total reproduction during 38 days was also counted. In results, the heart rate and body length of *D. magna* exposed to highest concentration of 6PPD-Q (1.0 mg L<sup>-1</sup>) significantly decreased after 21 days of exposure. In the case of locomotion, it was revealed that both 6PPD and 6PPD-Q promote the movement of *D. magna* exposed to the chemicals. Both chemicals were found to reduce the normal neonate incidence of water fleas in the high concentration exposure group, and it was observed that fertilized eggs that were not fully developed in the mother organisms were expelled from the body, resulting in a decrease in the reproductive rate of water fleas. Consequentially, long-term exposure of *D. magna* to 6PPD and 6PPD-Q induced decrease of individual growth, heart rate, and reproduction and increase of movements of organisms at high concentrations. This suggests that 6PPD and 6PPD-Q may adversely affect water flea populations and ecological health when continuously introduced to the aquatic environment. Acknowledgement: This work was supported by the financial support from the Korea Institute of Toxicology (KIT, KK-2309-03)

### **3.23.P-Th422 Fate, Effects and Ecosystem Interactions of 6-PPD-Quinone: A Freshwater Enclosure Study**

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Tire tread particles consist of rubber left behind on roadways by vehicle traffic that can be washed into waterways by rainfall. These particles contain various chemicals that enhance performance, durability, and safety of the tires. One of these chemicals, 6PPD, is an anti-ozonant, that breaks down into 6PPD-quinone (6PPDq) when exposed to solar radiation and oxygen. 6PPDq is toxic to certain fish species (e.g. coho salmon and brook trout) but there is a large range of sensitivity in even closely related fish (i.e. sockeye or chinook are insensitive). At the IISD-Experimental Lakes Area a whole ecosystem approach was used to investigate the fate and chronic sublethal effects of environmentally relevant 6PPDq concentrations in freshwater. A gradient of single-pulse exposures (5 - 100 µg/L), conducted using in-lake enclosures (~ 5,000 L), allowed us to better understand the effects of 6PPDq on lower trophic levels and how these indirect effects may contribute to fish mortality. Dissolved oxygen was measured using HOBO Dissolved Oxygen Loggers (5 min interval) and was lower (< 8 mg/L) in the highest two treatments (47.3 and 100 µg/L) compared to the control enclosures. Primary production was assessed using biofilm strips colonized for 3 weeks prior to sampling. There were no significant differences in chlorophyll-a production in the biofilm between treatments. Benthic invertebrate communities were assessed using Hester-Dendy substrate samplers and were dominated by chironomids in all treatments. There was significantly less colonization in the two highest treatments 3 days post-addition, but the invertebrates returned by day 8. Analysis of the corresponding emergent insect data is underway to help explain this anomaly. In addition to the in-lake experiment, we collected wild fathead minnow eggs to conduct in lab exposures using water from each enclosure 4 days post-6PPDq addition. The eggs were collected <24 hrs post-fertilization and monitored for 7 days in a temperature and light controlled room. There were no effects on growth or significant differences in the brain, eye, yolk sac or heart sizes relative to the total metabolic area of the larvae. There was, however, a significantly higher mortality rate and a

developmental delay in swimbladder inflation in the highest treatment (100 µg/L) compared to the controls. Additional results including water and sediment chemistry, zooplankton community structure, and emergent insect speciation will be presented.

### 3.24 Unveiling the Chemical Exposome: Insights From Human Biomonitoring and Its Influence on Adverse Health Outcomes

#### 3.24.T-01 Non-targeted screening for bisphenol related contaminants and restricted or unusual parabens in human milk

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Breast milk is a vital source of infant nutrition, yet it contains chemical contaminants that may pose health risks. Contemporary approaches to human milk biomonitoring predominantly rely on targeted methodologies, which are constrained in their capacity to identify emerging contaminants, suggesting the need for novel tools to improve their detection. In our study, non-targeted analysis (NTA) was applied to 194 human milk samples collected from the Vhembe district of Limpopo Province (South Africa), 193 samples collected from Pretoria (South Africa), and 207 samples from Montreal (Canada) to identify the presence of bisphenol derivatives, as well as other conjugated plastic-related unknowns. Liquid chromatography quadrupole time-of-flight mass spectrometry (Agilent Technologies) was used to detect 33 tentative bisphenol related unknowns by employing a customized database library of compounds. In total, the identity of twelve different bisphenol related compounds was confirmed using pure analytical standards. NTA was further applied to parabens in South African human milk samples, and the presence of 6 parabens confirmed using pure analytical standards. Notably, our study revealed the presence of several synthetic antioxidants as well as restricted or unusual parabens that have not been previously reported in human milk biomonitoring studies. This study highlights the importance of utilizing non-targeted analysis, demonstrating its potential to improve current human milk biomonitoring by uncovering a wide variety of unexpected or restricted compounds.

#### 3.24.T-02 Longitudinal Profiling of the Human Blood Chemical Exposome

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The temporal dynamics of the exposome are poorly understood, yet this is a critical factor at the design stage of long-term studies to minimize exposure misclassification and ensure statistical power for detecting multi-omics interactions or associations with disease. Within the Swedish SciLifeLab SCAPIS Wellness Profiling program, longitudinal profiling of the human blood chemical exposome was performed with repeated individual sampling (n = 6) from multiple individuals (n = 46) over 2 years.

Plasma samples (n = 276) were analyzed following a chemical exposomics protocol which combined solid-phase extraction for the removal of plasma phospholipid background with LC-HRMS (Orbitrap) multiclass targeted and untargeted analysis via data-independent acquisition (DIA) and a deconvolution-based open-source data processing workflow.

Data were analyzed integrating multivariate analysis, hierarchical clustering, mixed-effect models, and intra-class correlation coefficients (ICCs) to explore temporal trends and correlations of chemicals and metabolites, and to assess the individual variability and longitudinal stability of the chemical exposome.

The comprehensive analysis resulted in 129,547 unique untargeted features detected between ESI+ and ESI-, with targeted analytes comprising 0.04% of the dataset. The annotated dataset (0.4% annotation rate) included confirmed identities (Level 1) and confident structural candidates (Level 2a) for 343 environmental chemicals (11 subclasses), 162 endogenous metabolites, and 14 substances of ambiguous origin.

Chemical exposome and endogenous metabolites were classified based on their temporal variability (ICC) and incidence (detection frequency) across the population. Among the most common and stable components of the chemical exposome, PFAS substances were consistently detected in all individuals across all visits. In females, positive correlations were observed between testosterone and specific PFAS, while testosterone in males was negatively correlated with perfluorooctanoate (PFOA). These correlations suggest potential co-exposures and exposome-metabolome interactions, including endocrine disruption.

This study emphasizes the highly dynamic nature of the human blood chemical exposome. Recognizing novel exposures and exposome-metabolome interactions in individuals contributes valuable insight for advancing our understanding of the intricate interplay between environmental exposures throughout the lifecourse and human health.

#### 3.24.T-03 A Step Towards Assessing the Early Exposure to Per- And Poly-fluorinated Compounds (PFAS) in Childhood and Adolescence: A Human Biomonitoring Cohort Study

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Per- and poly-fluorinated substances (PFASs) have increased the attention as emerging, ubiquitous and persistent contaminants during the recent years. The first exposure to PFAS occurs in utero, and after birth, it continues via breast milk, food intake, environment (through air and dust) and consumer products containing these compounds. A chronic exposure to these compounds from early steps of life pose a relevant threat since it was found to contribute significantly to later health problems in adulthood.

In this scenario, in order to establish a possible causality between environmental or early exposure to PFAS and human health across the course of life, epidemiology plays a key role. In this regard, INMA (Childhood and Environment) research network was founded. Thus, a large survey of PFAS analysis in children plasma was performed, analysing N=623 samples from a Spanish cohort at 4, 8 and 14 years. For that, High Performance Liquid Chromatography coupled to a High-Resolution Mass Spectrometer (q-Orbitrap) was used, and up to 38 compounds were detected above the method limit of detection. The compound detection frequency results show a detection decrease according to age for some legacy PFAS. However, it is necessary to highlight that 26% of children would have levels above the threshold recommended by German Biomonitoring Commission for PFOA (2 µg/L). Likewise, the presence of shorter chain PFAS (PFBS, PFBA, PFPeA) nowadays considered replacement species had increased in more recent samples as well as compounds like GenX, ADONA or 9Cl-PF3ONS. The correlation analyses show a positive correlation between mother PFAS levels and levels at 4 years. Nevertheless, no correlation was found at 4 and 14 years. This might suggest that diet, breastfeeding and habits according to age may affect PFAS exposure patterns. In order to find which were the predictor factors associated with PFAS exposure, linear regression models were performed. The results show a positive association for mother levels and levels at 4 years, as well as, levels at 4 years and breastfeeding which may imply these two factors in PFAS early exposure. These findings suggest that the exposure pattern changes according to age. Indeed, a decreasing trend of the most persistent compounds, might be linked to the public health policies applied in recent years. Conversely, the detection of short-chain PFAS or emerging species in most recent samples may imply that the PFAS exposure paradigm is changing.

### **3.24.T-04 Advanced suspect screening approach for silicone wristbands and urine to reveal the early-life chemical exposome**

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Due to children's vulnerability, exposure to environmental chemicals during early-life represents a significant threat to developing chronic diseases<sup>1</sup>. A comprehensive understanding of chemical exposome is crucial to identify the exposures and devise effective intervention strategies. Target approaches that focus on determining specific classes of compounds overlook the health effects of dynamic chemical exposures. One of the main challenges faced is to develop a wide-scope screening approach to access a broader characterisation of the chemical exposome, and link environmental exposures and health<sup>2</sup>. Silicone wristbands (WBs) have recently emerged as an effective, low-cost, and child-friendly way of capturing volatile and semi-volatile organic compounds.

Our study aims to enhance the characterisation of the chemical exposome via a wide-scope suspect screening workflow based on HERMES<sup>3</sup>, using liquid chromatography and high-resolution mass spectrometry. 361 children (4-11 years old) living with smoker and non-smoker parents in the area of Tarragona (Northeastern Spain), home to the largest petrochemical site in Southern Europe, were sampled. HERMES is a molecular formula-oriented and peak detection-free method to generate sample-specific inclusion lists (IL). NORMAN Substance Database and in-house thirdhand smoke database (38147 unique molecular formulas) were used to create the ILs. In the WBs, 634 chemicals have been comprehensively annotated with a level 3, including tobacco smoke toxicants, personal care products, insect repellents, and plastic additives. From those chemicals, 29 were confirmed at level 2, whilst 3 were identified by standards. We also demonstrated that tobacco smoke exposure is underestimated and ubiquitous. Contrary to the reported 36% of children being directly exposed to tobacco smoke at home, we have determined that nicotine, cotinine, nicoteline and myosmine were present in 80% of the participants, indicating the relevance of outside home exposure.

Our work shows how a suspect screening workflow based on HERMES enhances the characterisation of the chemical exposome and confirm the potential impact of tobacco smoke exposure on children. The next step is the characterisation of urines to identify exposure biomarkers and metabolic alterations to find the association between air pollutants and health, to then compare the results from the two matrices.

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### **3.24.P Unveiling the Chemical Exposome: Insights From Human Biomonitoring and Its Influence on Adverse Health Outcomes**

#### **3.24.P-Th423 Assessing The Pregnant Women Chemical Exposome Through Serum And Placenta (Semi)Quantitative Analysis**

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The huge exposure to a myriad of pollutants and their introduction through diet, personal care products usage and daily activities have raised several questions related with their bioaccumulation in humans and their subsequent adverse health effects. One of the most concerning subpopulation is pregnant women, since newborns have a high risk associated with exposure, with possible outcomes such as low birthweight or preterm birth, among others. In order to estimate their chemical exposure, different tools have been employed, but the most informative one is through human biomonitoring. In our work (included inside of project AirNB), the main goal was to evaluate the occurrence of xenobiotics in 300 pregnant women from Barcelona, in both women' serum and placenta.

For the serum samples, a deproteinization with acetonitrile followed by centrifugation was made, while for placenta a tissue lysing combined with solid phase extraction (using wide-scope covering homemade cartridge) was applied. Procedural blanks (10%) were also included in the batch to account for possible contamination. Finally, all the samples were analyzed in an LC-HRMS (Q-Exactive) instrument and the data treatment was carried out with an in-house database of >700 contaminants. The quantification of the samples was done using matrix matched calibration curves, when the compound was included, and with an in-house developed semi-quantification tool based on ionization efficiency.

In serum samples, 149 chemicals were detected in at least 1 of the participants ranging from ng/L to mg/L while for placenta a total of 119 were detected. These chemicals included plasticizers (and their metabolites), tire additives, air pollutants, personal care products or flame retardants among others. We could observe 35 common chemicals between both matrices which gives information of the vital biological role of the placenta as a chemical barrier for foetus wellbeing. However many other chemicals detected in the serum (and not in placenta) might be permeating the barrier, posing a potential threat to the foetus and its development. This information will be used for the analysis (currently ongoing) of paired cord blood samples. These results will be used in collaboration with epidemiologists involved in Barcelona Life Study Cohort to identify metabolic dysregulation or health outcomes linkages caused by the presence of these chemicals.

### **3.24.P-Th424 Potential Association of Endocrine-Disrupting Chemicals with Frontal Fibrosing Alopecia: A Case-Control Study**

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Frontal Fibrosing Alopecia (FFA) is the leading cause of scarring alopecia, especially affecting postmenopausal women. Although the exact causes of FFA remain unclear, genetic and hormonal factors are suspected to be involved. Recent research suggests that environmental factors, particularly endocrine-disrupting chemicals (EDCs), may contribute to FFA development by interfering with natural hormones and impacting long-term health.

Our research focuses on the potential impact of EDCs in FFA development. For that purpose, we conducted a *proof-of-concept* case-control study involving 10 FFA patients and 10 age-matched controls, and collected blood, urine, and hair samples for analysis. Serum samples were deproteinized with acetonitrile. Urine samples underwent centrifugation to remove solids, and Captiva cartridges were employed for endogenous chemical filtration. Hair samples were cleansed of surface contaminants with dichloromethane and methanol, ground with bead beating and EDCs were extracted using MeOH. The analysis was performed using liquid chromatography coupled to high-resolution mass spectrometry (LC-QTOF).

Among the 48 EDCs analyzed, 35 were detected, with the highest number of compounds found in hair samples (63%), followed by urine (42%) and serum (27%). Parabens and flame retardants were consistently present in all matrices. The concentration range in hair was notably higher than in the other two matrices, which may be because hair provides information on long-term exposure. To assess differences between FFA group and controls, the Mann-Whitney U test was performed. Statistically significant differences ( $p < 0.05$ ) were observed for mono(2-ethyl-5-oxohexyl phthalate) (5-Oxo-MEHP) and triphenyl phosphate (TPhP) in urine samples, and a noticeable trend towards higher concentrations in FFA cases compared to controls was observed in hair and urine, suggesting a potential association with FFA. Serum samples, possibly affected by the limited sample size and the number of detected compounds did not exhibit the same trend.

In conclusion, our study highlights the importance of using diverse biological samples for understanding the chemical exposome. While 5-Oxo-MEHP and TPhP in urine of FFA group is significantly higher ( $p < 0.05$ ) than in the control group, a broader trend towards higher concentrations in FFA cases was notable in hair and urine matrices. It is important to note that this study is a *proof-of-concept* and results should be interpreted cautiously.

### 3.24.P-Th425 Nontarget and Multi-Class Target Chemical Exposomics in Human Plasma by Lipid Removal and Large Volume Injection GC-HRMS

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Research on the human chemical exposome depends on improved analytical methods, which are still evolving to meet the practical challenges of sensitivity and a wide chemical space. Only 5% of the 350,000 chemicals in global commerce have ever been analyzed in environmental media, primarily by targeted methods. Recent advances have been reported for multi-class target or nontarget chemical exposomics in human plasma by gas chromatography (GC-) with Orbitrap high resolution mass spectrometry (HRMS), however Orbitrap technology can suffer from low sensitivity for trace analytes in complex matrices due to the auto gain control (AGC) function, and co-extracted lipids may lead to instrumental fouling and high demands for maintenance. We hypothesized that a GC-Orbitrap HRMS chemical exposomics workflow for plasma could be enhanced by effectively removing major lipid interferences at the extraction stage, thereby also enabling large volume injection of the extracts for increased method sensitivity. In this study, a representative list of 103 target analytes from 6 chemical classes were selected for method development, including tests of various lipid removal steps (e.g. liquid-liquid and solid-phase extractions) and different instrumental parameters. Here a simple and potentially scalable liquid-liquid extraction protocol was developed and validated for GC analysis of small plasma sample volumes while optimizing for lipid removal, high-recovery and sensitivity of priority target analytes. The validated median method quantification limit (MQL) was 0.08 ng/mL (range 0.005–4.83 ng/mL) for 200 µL plasma samples. In individual adult plasma (100 µL, n=32), 52 target analytes from 5 classes were detected. Of these, 25 were above MQL and were quantified, 8 of which also showed significant increasing or decreasing temporal trends. In nontarget analysis, a total of 992 molecular features were detectable at least once among all individual plasma samples, and 81 high-confidence annotations were made. Ongoing application of the method to a larger longitudinal cohort will also be discussed.

Keywords: Human exposome, gas chromatography (GC), Orbitrap, high resolution mass spectrometry (HRMS), Chemical exposure, Plasma

### 3.24.P-Th426 Unveiling the Chemical Exposome of Brain Cancer: A Proof of Concept

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Diffuse gliomas are a highly heterogeneous and aggressive brain tumours with poor prognosis and survival and few established risk factors. Environmental exposures are suspected in the pathogenesis of these tumours; however, results of existing studies are limited and inconsistent, particularly for exogenous organic chemicals, with no available characterization of the chemical exposome of these tumours. Also, better understanding of phenotypic differences in tumour types is needed in order to improve clinical decision making and provision of personalised treatment recommendations. In this proof-of-concept study we analysed 33 glioblastoma samples (Bellvitge Glioma Cohort (BGC), Spain, 2005–present), including 16 methylated and 17 non-methylated tumours combining HRMS-based wide-scope target and suspect strategies [1]. Forty-six exogenous chemicals were identified in the tumour tissue samples (31 confirmed with standard) including a variety of industrial chemicals (e.g. plastic additives or perfluorinated compounds), personal care products and pharmaceuticals. Our findings provide novel evidence on the presence of these chemicals in brain tissue, highlighting the need for comprehensive evaluations of their potential effects in the tumour pathogenesis. Finally, after applying metabolomics methods we observed clear differences in the profiles of endogenous chemicals among the studied glioma subtypes, and identified possible biomarkers. These chemicals have potential to be determined in a non-invasive manner, either by LCHRMS-based blood analysis or using complementary techniques (proton magnetic resonance (1H-MRS)). These are inspiring results since methylation is a strong independent predictor of survival as well as tumour response to chemotherapy for glioblastoma. Indeed, its non-invasive and pre-surgical determination would have a major impact on patient management. Our preliminary data is suggestive for the potential of nontargeted exposome methods to find new valuable biomarkers for diffuse gliomas diagnostic and prognostic stratification.

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### 3.24.P-Th427 Holistic determination of the prenatal exposome for a comprehensive overview of placental barrier

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The complex interplay between pregnancy and environmental exposures, particularly to Endocrine-disrupting Chemicals (EDCs), unveils a compelling avenue for research in maternal-fetal health. The distinctive biological changes that transpire during pregnancy render women uniquely susceptible to the disruptive effects of EDCs. In this proof-of-concept investigation, we embarked on a pioneering endeavor employing a comprehensive and efficient analytical approach, tapping into the full potential of HRMS-based strategies. Our primary objectives were to (1) conduct a thorough characterization of the human chemical exposome in pregnant women and (2) elucidate the physicochemical properties of chemicals capable of traversing the placental barrier, thereby potentially compromising the fetal environment. We applied a wide-scope target analysis encompassing over 2000 chemicals by means of a Q-Exactive Q-Orbitrap instrument coupled to UHPLC to paired samples of urine, maternal blood, cord blood, and placental tissue sourced from 23 pregnant women within the population-based INSULIN mother-child cohort (Ref. CEim 225/2020). Our results unveiled the presence of 81 distinct chemicals observed in at least one of the sampled biofluids. Specifically, our study revealed that 45 of these chemicals demonstrated the ability to traverse the placental barrier, as evidenced by the presence of 10 food components, 5 pharmaceuticals, 8 personal care products, 8 plasticizers, 1 pesticide, 4 flame retardants and 9 industrial chemicals. Inverse correlations were observed between placental transfer rates and LogP ( $\rho = -0.438$ ;  $p < 0.001$ ), molar refractivity ( $\rho = -0.485$ ;  $p = 0.003$ ), volume of van der Waals ( $\rho = -0.386$ ;  $p = 0.003$ ), number of heavy atoms ( $\rho = -0.360$ ;  $p = 0.005$ ), number of hydrogen (H) ( $\rho = -0.361$ ;  $p = 0.005$ ) and carbon (C) ( $\rho = -0.407$ ;  $p = 0.001$ ), and eccentricity ( $\rho = -0.332$ ;  $p = 0.01$ ). In conclusion, pregnant women face extensive exposure to a myriad of chemicals, with particular concern directed towards compounds exhibiting heightened hydrophilicity, smaller size, compact structure, and reduced complexity, which possess an elevated likelihood of traversing the placenta, thereby raising concerns about their potential impact on fetal development. Implementing urgent measures tailored to substances characterized by properties conducive to placental crossing is imperative to safeguard the health of future generations

### 3.24.P-Th428 Advanced HRMS-Based Strategies for Profiling and Semi-Quantification of Exogenous Chemicals in Human Urine.

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Chemical substances permeate our daily lives through various exposure routes, prompting the need for comprehensive assessment tools. Human biomonitoring (HBM) traditionally relied on targeted screening methods employing triple quadrupole instruments and a limited set of chemicals. Advances in high-resolution mass spectrometry (HRMS) have expanded the scope, enabling wide-scope target, suspect, and non-target analyses within acceptable detection limits. Despite these advances, establishing reliable sample treatment protocols remains crucial for broad-range chemical analysis. This research aimed to validate a methodology utilizing HRMS-based approaches for precise profiling of exogenous chemicals and associated metabolites in urine samples. We assessed five extraction protocols, spanning diverse chemical classes such as pharmaceuticals, plastic additives, personal care products, or pesticides. Our assessment considered extraction recoveries, linearity, matrix effect, sensitivity, and reproducibility. The most effective protocol underwent thorough validation with a set of 90 chemicals and was applied to 10 real human urine samples via wide-scope target analysis, covering over 2,000 chemicals. Using an ionization efficiency-based model, we successfully identified and semi-quantified 36 chemicals, demonstrating the robust performance of the methodology. Importantly, our findings challenged the necessity of a deconjugation step, a traditionally labor-intensive and time-consuming process.

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### 3.24.P-Th429 Biomonitoring of Different Endocrine-Disrupting Chemicals Metabolites in Urine Samples

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A multitude of chemicals and contaminants enter the human body, through various exposure pathways, predominantly via inhalation, ingestion, or dermal contact. A common example is the endocrine-disrupting chemicals (EDCs). These chemicals (e.g. phthalates, organophosphate esters (OPEs), and parabens) mimic endocrine hormones, which can affect the normal physiological mechanisms of human beings, even at low concentrations, leading to some health issues. So the biomonitoring of these compounds in human samples is crucial to understanding the possible adverse effects that global exposures (including all pathways) could cause on human health. Of the many matrices available for human biomonitoring, urine samples stand out due to their high sample volume availability, easy collection, and non-invasive nature.

This study focused on developing and validating a rapid analytical methodology to have a successful approach for the biomonitoring of diverse EDCs including eight OPEs, five phthalates, and three parabens. To set up the method, specific aspects of the extraction procedure were rigorously evaluated for their impact on sensitivity and matrix effect. Furthermore, a systematic optimization of both chromatographic and mass spectrometry conditions was implemented, utilizing ultra-high-performance liquid chromatography (UHPLC) coupled with tandem mass spectrometry (QTOF). Additionally, for later real sample analysis, creatinine concentrations in urine were determined to correct metabolite concentration levels due to urine dilution.

The optimized method exhibited favorable analytical performance, including acceptable recoveries ranging from 46 to 99%, with acceptable relative standard deviations ( $RSD \leq 20\%$ ), and low LODs and LOQs. This method was finally applied to biomonitoring 16 EDCs in 44 urine samples; 29 samples were obtained from teenagers (ages 13-16), and 15 from workers in an e-waste recycling company (e-waste RC), considered to have higher exposure due to their occupational activities. EDCs metabolites were identified in all urine samples. Notably higher concentrations of EDC metabolites were observed in samples from workers of the recycling company than in teenager samples, particularly for phthalate metabolites, commonly found in their occupational environment. The results obtained from the urine sample analysis were used to calculate the estimated daily intake and the corresponding risk associated with this exposure, for both groups of citizens.

### **3.24.P-Th430 Wide-scope Target and Nontarget Profiling of the Airborne Chemical Exposome using Polydimethylsiloxane (PDMS) Passive Samplers**

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Air pollution is a major cause of premature mortality worldwide, and exposures to various airborne substances in our homes, workplaces, or in ambient air, have been implicated in disease. Nevertheless, the chemical composition of the air we breathe is poorly characterized. To support next generation research on the airborne exposome, we therefore aimed to develop an inexpensive and easily deployable air sampler that would be suitable for nontarget analysis, and which could simultaneously collect (semi-) volatile organic compounds and particulate matter.

To achieve this, we recently reported on the synthesis and nontarget analysis of polydimethylsiloxane (PDMS) foam disks for passive sampling of airborne contaminants. In the initial investigation, PDMS foam deployed indoors for 1 to 3 months, and analyzed by gas chromatography (GC-) and liquid chromatography (LC-) coupled to high-resolution mass spectrometry (HRMS), revealed accumulation kinetics for polar and non-polar substances that suggested effective uptake of both the gas- and particulate-phase. In the current work we further investigate the sampling properties of PDMS foam disks obtained from a commercial supplier, and report on the optimization and quantitative validation of LC-HRMS and GC-HRMS methods for over 200 target analytes from 20 chemical families with diverse physicochemical properties.

Two pilot studies followed method validation. The first study (indoor and outdoor, 6 weeks) assessed chemical uptake and its correlation with particles adsorbed onto the foam surface. Using microscopy, outdoor foams deployed for 3 weeks and 6 weeks averaged 136 and 260 particles  $\text{mm}^2$  respectively, showing a significant increase and linear sampling capacity for particles ( $R^2 = 0.944$ ,  $r = 0.972$ , and  $p\text{-value} = 0.03$ ). The second study (indoor, 8 weeks) evaluated reproducibility between replicates using quantitative (target) and qualitative (nontarget) approaches. Among target analytes, plasticizers (e.g. phthalates), various industrial chemicals (e.g. 4-nitrophenol), insecticides (e.g. DEET), and flame retardants (e.g. OPFRs) were most frequently detected. Nontarget analysis further allowed confident annotation (level 2) of additional industrial and commercial substances not in the target list, in addition to natural products and plant volatiles. Within the framework of the Horizon Europe INQUIRE project, PDMS foam disks are now being deployed in over 200 homes across 8 European countries.

### **3.24.P-Th431 Exposure level to legacy phthalates and alternative plasticizer metabolites in urine during pregnancy and delivery**

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Phthalates have been widely used as plasticizers to soften plastics. When exposed to the human body, they interfere with or disrupt the action of hormones, acting as endocrine-disrupting chemicals (EDCs), and have been reported to exhibit reproductive toxicity, including infertility. Consequently, international regulations have been imposed on the use of phthalates, leading to the development of alternative plasticizers to evade regulations. However, there is limited information on the toxicity of alternative plasticizers and data on human exposure monitoring are lacking. Pregnant women are sensitive to chemical substances, and EDCs can be transmitted to the fetus through the placenta. This study aimed to assess the levels of legacy phthalate and alternative plasticizer metabolites in the urine of pregnant women during pregnancy and delivery.

Sixteen metabolites of legacy phthalates and 20 metabolites of alternative plasticizers were selected as target analytes. Urine samples from the “DearMom cohort” collected in Korea in 2022 (first trimester (n=16), second trimester (n=42), third trimester (n=37), and at delivery (n=27)) were analyzed. Samples were processed by enzymatic treatment, followed by solid-phase extraction, and were quantitatively analyzed using LC-MS/MS.

The results of the urine sample analysis revealed that the detection rates of 10 metabolites of legacy phthalates and eight metabolites of alternative plasticizers were above 70%. As a result of comparing urine concentrations (median) by quarter of pregnancy (1st, 2nd, 3rd trimester, and at birth), MCPP (0.635, 0.561, 0.988, 0.130ng/mL) and DEC (3.860, 4.600, 7.910, 0.049) ng/mL were significantly lower in urine concentrations at delivery than during pregnancy (p<0.05). MEHP (0.862, 1.340, 0.988, and 4.470 ng/mL) and OH-MINCH (0.133, 0.149, 0.140, and 1.400 ng/mL) levels remained at similar levels during pregnancy and then increased at birth (p<0.05). Additional research is needed to investigate the association between phthalate and alternative plasticizer levels and the characteristics of mothers (age, pregnancy experience) and infants (birth size, placental pathology).

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### 3.24.P-Th432 Lipidomic Changes and Toxicity Induced in Human Lung Cells by a Mixture of Water Disinfection byproducts

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Complex mixtures of disinfection by-products (DBPs) are present in disinfected waters, but their mixture toxicity has been rarely described. Apart from ingestion, DBP exposure can occur through inhalation, which may lead to respiratory effects in highly exposed individuals. However, the underlying biological mechanisms have yet to be elucidated. This study aimed to investigate the toxicity of a mixture of 10 DBPs, including haloacetic acids and haloaromatics, on human alveolar A549 cells by assessing their cytotoxicity, genotoxicity, and impact on the cell lipidome. A DBP mixture up to 50 µM did not affect cell viability but induced the generation of reactive oxygen species (ROS) up to 3.5-fold, and increased the frequency of micronuclei formation. Exposure to 50 µM DBP mixture led to a significant accumulation of triacylglycerides (TGs) and a decrease of diacylglycerides (DGs) and phosphatidylcholines (PCs) in A549 cells. Lipidomic profiling of extracellular vesicles (EVs) released in the culture medium revealed a marked increase in cholesterol esters (CEs), sphingomyelins (SMs), and other membrane lipids. Overall, these alterations in the lipidome of cells and EVs may indicate a disruption of lipid homeostasis, potentially contributing to the respiratory effects associated with DBP exposure.

### 3.24.P-Th433 "Exposure Levels of Environmental Phenols in the Urine of Pregnant Women during Pregnancy and Childbirth"

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Environmental phenols are one of the major endocrine-disrupting chemicals (EDCs) that act as environmental pollutants. When they are absorbed into the human body, they can disrupt natural hormones. Exposure to environmental phenols during pregnancy can lead to conditions such as premature birth, fetal growth retardation, and low birth weight. Pregnant women are particularly vulnerable to the adverse effects of environmental pollutants, which can harm both the mother and fetus. Therefore, this study aims to investigate the levels of environmental phenol exposure during pregnancy and childbirth by analyzing urine samples.

The target analytes are 28 compounds, including 7 parabens, 4 paraben metabolites, 2 antimicrobials, 4 benzophenones, and 11 bisphenols. Urine samples of the “DearMom cohort” were collected quarterly from pregnant women in Korea in 2022, 16 in the first quarter, 42 in the second quarter, 37 in the third quarter, and 27 during childbirth. The samples were stored at -70°C until analysis. Preparation of urine samples was performed using liquid-liquid extraction. Quantitative analysis was carried out using UPLC-MS/MS.

The results indicate that 7 out of 28 analytes had detection rates above 60%. The median values of 7 compounds are as follows; MeP 66.50 ng/mL, EtP 41.25 ng/mL, 4-HB 697.00 ng/mL, 3,4-DHB 74.25 ng/mL, OH-MeP 1.77 ng/mL, OH-EtP 0.43 ng/mL, BPS 0.31 ng/mL. Among the 7 analytes, MeP, 4-HB, 3,4-DHB, OH-MeP, and BPS showed significant differences across quarters (p<0.05). The t-test results showed increasing levels of 4-HB, OH-MeP, and BPS during the third quarter, while MeP increased at childbirth. In contrast, 3,4-DHB decreased at childbirth (p<0.05). Additional research on environmental phenol exposure is necessary to effectively manage health risks for pregnant women and fetuses.



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### 3.24.P-Th434 Identification of exogenous organic compounds in blood plasma, seminal plasma and urine, and their association with human semen quality

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Decreased semen quality has been described in different industrialized regions worldwide. Published studies suggest that the exposure to a growing number of synthetic chemicals could be responsible of a poor semen quality and contribute to the male fertility decline in the last decade. Hence, it is important to deeply characterize male's chemical exposome. The present study is aimed at identifying the presence of exogenous chemicals in urine, blood plasma and seminal plasma, and explore their associations with semen quality parameters. Hence, we applied a novel LC-HRMS method for an exposome scale profiling of >2000 organic chemicals in these matrices from participants (n=48) to the Spanish LED-FERTYL cohort. Results revealed a total of 49, 48 and 33 chemicals detected in at least one sample of urine, semen or blood plasma, respectively. Among them, we have found pharmaceuticals, industrial chemicals, biocides and food related chemicals. Five of them were simultaneously found over 25% urine and blood plasma samples, highlighting the high positive correlations for the herbicide quinmerac and caffeine ( $r=0.84-0.82$ ) followed by cyclamic acid( $r=0.51$ ). Likewise, four compounds were also simultaneously detected in urine and seminal plasma, highlighting the high positive correlations between matrices for the caffeine and quinmerac( $r=0.80-0.75$ ) followed by the theobromine( $r=0.31$ ). Some surfactants, methylparaben, nicotinamide, caffeine and quinmerac were concurrently detected in blood plasma and seminal plasma, also exhibiting strong between-matrix correlation for quinmerac and caffeine ( $r=0.89-0.78$ ). We identify physical activity, body mass index, age, use of solvents and smoking as variability factors for several compounds. The preliminary analysis with semen quality revealed negative bivariate correlations between theophylline and theobromine in urine with sperm motility stands out. In semen, a negative association between nabumetone and sperm concentration was found, and in plasma, the negative association between nicotine and sperm vitality also stands out. These preliminary analysis support that synthetic and other exogenous chemicals are widespread in human biological matrices, including seminal plasma. Further analyses are still ongoing to characterize the potential impact on seminal parameter standard. The analysis of exposure determinants will help to identify modifiable factors to reduce the male's chemical burden and associated decline of semen quality

### 3.24.P-Th435 Korean National Biomonitoring Programs and Comparative Toxicogenomics Database Analysis Reveals AOP of Atopic Disease by DEHP Exposure: Epidemiology and Toxicology Combined Approach

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Biomonitoring programs have been widely adopted in the field of environmental health, and biological data obtained from these programs have been organized based on biobank systems and used for various purposes. Here, using six Korean national biomonitoring programs (i.e., MOCHE and KorEHS-C, KoNEHS (Cycle I, II, III and IV)), the temporal trends of DEHP exposure from 2009 to 2020 and its association with allergic diseases (i.e., atopic dermatitis, asthma, and allergic rhinitis) in the Korean general population were identified. In our analysis, the urinary levels of DEHP metabolites, MEHHP and MEOHP, exhibited a distinct decline as participants aged, with a noticeable reduction, particularly in young children (3–6 years) since 2015. This recent decrease in DEHP exposure may be due to the implementation of the K-REACH Act (2015) as well as product safety policies. The concentration of urinary metabolites was used to derive EDI using reverse dosimetry with a PBPK. The highest EDI was observed in the toddlers (1–2 years), followed by adolescents (13–18 years) and the EDIs remained comparable to adults and the elderly. Then, the relationship between urinary concentrations of three different DEHP metabolites of MEHHP, MEOHP, and MECPP and allergic diseases among children aged 6–11 years in Korea from 2018 to 2020 was examined. Patients with diagnosed atopic dermatitis in the Korean children (6–11 years) had higher urinary concentrations of both creatinine and specific gravity-corrected DEHP metabolites ( $p<0.05$ ). In boys, increased DEHP exposure was positively associated with atopic dermatitis (Odds ratios 1.625–1.938) after adjusting age, BMI, and house income. Based on this, AOP of DEHP leading to atopic dermatitis has been developed by integrating five types of curated interactions within the CTD and KEs from AOPwiki. Our study presents a novel framework for integrating multiple databases in both epidemiology and toxicology. This approach helps unexplored knowledge gaps that exist within individual databases.

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### 3.24.P-Th436 Multi-target analysis and suspect screening of exposome-related xenobiotics in human follicular biofluid

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The ovarian follicular fluid is a human matrix of great interest in determining infertility, which is a worldwide public health problem. Although the number of studies to date is scarce, follicular fluid could act as a reservoir for many of the xenobiotics that might be directly related to infertility. Therefore, robust analytical methods able to determine a wide variety of exposome-related xenobiotics in that matrix are compulsory. In the present work, a target method for the analysis of almost 200 xenobiotics (industrial chemicals, personal care products, biocides, and pharmaceuticals) in the follicular fluid was developed. Regarding the extraction, a salt-assisted liquid-liquid extraction (SALLE) using acetonitrile was optimized in terms of sample volume (0.5 and 1.0 mL) and nature of the salt (Na<sub>2</sub>SO<sub>4</sub> and MgSO<sub>4</sub>). Moreover, several clean-up strategies were evaluated, such as, protein precipitation by centrifugation, selective filters for the removal of lipids and proteins, and solid-phase extraction using normal- and reverse-phase sorbents. Finally, the extracts were analysed by liquid-chromatography – high resolution mass spectrometry (UHPLC-HRMS/MS). The optimal conditions were selected in terms of recoveries, matrix effects, repeatability, and number of detected analytes and resulted in 0.5 mL of sample and MgSO<sub>4</sub> for the extraction, followed by protein precipitation by centrifugation. In fact, more than 90 % of the analytes were recovered with absolute recoveries ranging between 13 % and 65 % with relative standard deviations (n=3) lower than 30 %. Regarding the matrix effect at detection, signal suppression was observed. The optimal protocol was validated at 2 concentration levels (5 and 30 ng/mL) on two different days and the following figures of merit were calculated: instrumental limits of quantification, linearity ranges, instrumental repeatability, procedural limits of quantification (pLOQ), accuracy (absolute and apparent recoveries), and methods' precision (intra- and inter-day repeatability). All in all, satisfactory figures of merit were achieved considering the complexity of the matrix and the wide variety of analytes included. In addition, suspect screening was performed using a pooled sample (n=28) detecting several xenobiotics and metabolites at different confidence-levels.

### 3.24.P-Th437 Determination of Aflatoxin B1 and M1 in human breast milk

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Human milk constitutes a wealth source of nutritive compounds such as lipids, carbohydrates, proteins, amino acids, minerals, vitamins and non-nutritive elements, like enzymes, immunoglobulins, nucleic acids, hormones, growth factors, and cells (including macrophages, lymphocytes, and epithelial cells) that contribute to the infant's development and well-being (Marchant et al., 2018). Despite the numerous benefits associated to human milk, some toxic compounds that occur during the food chain can be detected in human milk (Lindeman et al., 2021). Breastfed infants are more vulnerable and sensitive to toxic compounds due their lower capacity to excrete compounds and body clearance. Aflatoxins (AFs) are carcinogenic, mutagenic, and immuno-suppressive mycotoxins, that have been correlated with liver cancer (Marín et al., 2013). Aflatoxin M1 (AFM1), constitutes on of the principal hydroxylated metabolite of aflatoxin B1 (AFB1), detected in milk, being considered a biomarker of AFB1. This detoxification product is originated as result of AFB1 metabolism and shows about 10% of mutagenicity compared to its precursor (Marchese et al., 2018). In the present study a method for the analysis of AFB1 and AFM1 in samples of mature breast milk collected one month after delivery from 57 mothers was developed. The extraction method consisted in a first step of defatting with hexane, then the extraction was carried out with H<sub>2</sub>O and acetonitrile and a mixture of MgSO<sub>4</sub>, NaCl, DSCPH and TSCPH salts. The determination has been carried out by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). The results revealed that AFB1 only was detected in 5% of samples at levels above LOQ with mean concentration in positive samples of 4,5 µg/L. Regarding AFM1, 8 of 57 (14.03%) breast milk samples resulted positive and the levels in positive samples were above the limits established by the legislation in raw milk (0.05 µg/Kg) (EC, 2023/915). Despite some positive results, it should be highlighted that the major part of samples were not contaminated. More data is needed for a more accurate infants risk assessment.

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## Track 4. Ecological and Human Health Risk Assessment of Chemicals, Mixtures and Stressors and Risk Mitigation Strategies

### 4.01.A Advancements in Bioremediation and Phytoremediation for Addressing Persistent and Emerging Pollutants in Contaminated and Degraded Ecosystems

#### 4.01.A.T-01 Soil bioresilience through bioavailability reductions: engineering components and policy challenges

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In a world facing global change, the strategic value of soil cannot be underestimated. The extensive costs associated with remediating polluted sites, compounded by the need for financial recovery from the COVID pandemic and Ukraine war, demand innovative solutions of site remediation. Our solutions prioritize sustainability principles, aligning with the UN Sustainable Development Goals and the European Green Deal. Nature-based solutions (NBS), such as bioremediation and phytoremediation, offer promising avenues for soil restoration. Traditional NBS approaches focused solely on total pollutant removals have proven unpredictable and often fall short of legislative remediation goals. With a focus on hydrophobic organic pollutants and bioremediation, this overview presentation will explore the paradigm shift towards addressing bioavailability reductions, surpassing mere total pollutant removals, to reach soil bioresilience, a new term reflecting the successful restoration of soil functions based on the management of pollutant bioavailability. We will examine the existing bioavailability concepts and methods, options for their application and standardization, as well as nature-based engineering components for addressing bioavailability reductions. Our nature-based engineering components were demonstrated to enhance bioremediation performance and reduce risks at various levels of bioavailability processes, encompassing interactions between contaminants and soil, their transportation, and biological processing. The focus will extend to our research on hydrophobic organic pollutants and bioremediation, unveiling strategies such as the precise targeting of slow-desorption pollutant fractions using (bio)surfactants, fine-tuning the deposition and motility of microbial degraders, co-mobilizing non-motile inoculants, and effectively capturing pollutants through plant-biochar arrangements, showcasing their potential to transform soil remediation practices. The contribution will also encompass insights into how addressing bioavailability challenges may contribute to prominent European initiatives, including the Green Deal, thereby empowering missions related to soil health and food security, the action plan for zero pollution, and the chemicals strategy for sustainability.

#### 4.01.A.T-02 DNA-SIP combined with metagenomics unveil bacterial communities and mechanisms involved in azaarene biodegradation in PAH-contaminated soils

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Nitrogenated-containing polycyclic aromatic heterocycles (N-PAHs), also referred to as azaarenes, are pollutants found in contaminated soils along with polycyclic aromatic hydrocarbons (PAHs). Investigations applying high-resolution analytical methods have unveiled a hitherto unrecognized high abundance and diversity of azaarene isomers in PAH-contaminated soils. The biodegradability of these compounds was isomer-selective, as a function of their molecular weight, degree of methylation, and the position of the nitrogen group.

In this study, an analysis of the microbial community response to distinct azaarene congeners in creosote-contaminated soil revealed that the differential susceptibility to biodegradation could also be associated with distinctive microbial communities involved in the degradation of specific azaarenes. To elucidate the mechanisms involved in azaarene assimilation, a series of experiments were conducted on soil slurry microcosms using acridine as a model three-ring azaarene. High-performance liquid chromatography-mass spectrometry (HPLC-MS) was employed to assess biodegradation kinetics, demonstrating effective acridine removal after a 5-day incubation period. To identify the microbial populations and functions involved in acridine assimilation, DNA-stable isotope probing was performed using uniformly labeled <sup>13</sup>C-acridine.

Analysis of the 16S rRNA clone library of <sup>13</sup>C-enriched DNA identified a member of *Sphingobium* as the primary acridine degrader. Shotgun metagenomic sequencing provided functional insights into the biodegradation process, enabling the reconstruction of the acridine metabolic pathway in soils. Reconstruction of metagenome-assembled genomes (MAGs) resulted in the assembly of a single MAG. Functional analysis of this MAG revealed nutritional auxotrophies for specific vitamins and amino acids. Based on this information, a molecular-directed isolation strategy was implemented to obtain the first bacterial isolate with the ability to grow on acridine.

#### 4.01.A.T-03 Bioremediation of soils contaminated with PFAS: challenges, successes and future directions

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Per- and polyfluoroalkyl substances (PFAS) are a class of anthropogenic organofluoride chemicals, widespread in industrial

and consumer applications, well known for their unique and advantageous properties, such as their surface activity, thermal and chemical stability. However, the combination of their extensive use, bioaccumulative nature and adverse effects to the environment and human health has put them in the focus of scientific research as well as regulators with the aim to develop suitable remediation measures for polluted locations.

PFAS resistance to degradation is attributed to the C-F bond. Despite being commonly cited as the strongest bond, the strength of C-F bonds in PFAS molecules varies significantly. PFAS structures, analyzed using density functional theory, reveal higher bond dissociation energies for primary C-F bonds than secondary ones, with longer fluoroalkyl chains exhibiting lower dissociation energies.

This work aims to provide an overview on the current state of art of the most effective PFAS soil bioremediation strategies employed until now. Although popularly known as “forever chemicals”, based on recently published articles, microorganisms isolated from PFAS polluted environment can have capacities for degradation of these compounds. Some of these microorganisms are: *Acidimicrobium* sp. strain A6, *Pseudomonas parafulva* strain YAB1, *Pseudomonas plecoglossicida* 2.4-D, White-rot fungus, *Phanerochaete chrysosporium*, *Pseudomonas butanovora* and *Pseudomonas oleovorans*. For the microbial communities isolated from Antarctic marine habitats, activated sludge, soil and sediment polluted with PFAS, similar capacity was confirmed.

PFAS biodegradation involves enzymes that remove fluorine atoms through oxidation or reduction of the C-F bond. While experimental evidence has illustrated the biotransformation of some PFAS, the proposed degradation mechanisms differ significantly, and a confirmed biodegradation pathway has yet to be established. In addition, although the bioremediation of PFAS is still in the research and development phase, it should be emphasized that some microbes can break the C-F bond in naturally produced fluoroacetate and fluorobenzoate via hydrolytic defluorination. Knowing that PFAS have been introduced to the environment seventy years ago, one would expect that nature had already developed microbial mechanisms to degrade these chemicals. The hunt for the most potent species and consortium is open.

#### **4.01.A.T-04 Metagenomic Study on Nature-Based Solutions for Bioremediation: Investigating a Bioreactor with Biochar and Polyhydroxyalkanoates as Biomaterials for Biological Anaerobic Dechlorination**

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The use of bio-based materials in bioremediation technologies is appealing for the restoration of contaminated sites. Some studies have demonstrated the effectiveness of the polyhydroxybutyrate (PHB), a bio-synthesized polyester biodegradable in the environment, as a slow-release electron donor to sustain reductive dechlorination (RD) processes [1]. Also the organic waste-derived biochar (BC), produced by the pyrolysis of biomass and used for contaminants' adsorption, is capable to accelerate the electron transfer in several bioprocesses [2]. PHB and BC have been successfully employed in engineered systems for the treatment of chlorinated solvents [3]. Here we describe the composition and metabolic features of the microbiome established in a column bioreactor that couples PHB and BC as bio-based materials to prompt the RD of TCE-contaminated groundwater. In line with the kinetic performances of the reactor, *Dehalococcoides mccartyi* (*Dhc*) established both in the PHB (1.46E+06 16S rRNA gene copies/g) and BC (5.51E+08 16S rRNA gene copies/g) reactive zones, suggesting for the first time BC as a vehicle for biological RD and a support for the growth of *Dhc*. In addition, the metagenomes of the PHB and BC reactive zones have been analyzed to investigate the central mechanisms occurring in the biofilm growing on these biomaterials. *Clostridiaceae* (*Clostridium*, 48% of total ASVs) and *Victivallaceae* (15% of total ASVs) families, primarily involved in the fermentation processes, dominated the PHB zone. The results of the genomic-centric analysis highlighted the main metabolic features of the PHB and BC biofilms of the column system and will be discussed in the presentations.

#### **4.01.A.T-05 PHYTOREMEDIATION OF AGRICULTURAL AREAS CONTAMINATED BY HYDROCARBONS**

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In 2015, in an agricultural area close to Milano, oil spills occurred causing the release of fuel in the soil. A detailed (hydro)geological, chemical, pedological and microbiological characterization was performed to design the best remediation technologies. Based also on the site typology and size and on contamination degree and depth, bioremediation and phytoremediation were selected as the best options in terms of both environmental and economic feasibility and sustainability with the aim of preserving the "soil resource" and avoiding any action that could negatively affect the ecosystems. In the 1<sup>st</sup> site, Biopile and Phytoremediation pilot-scale tests were carried out. The full-scale phytoremediation of this site started in 2023. In the 2<sup>nd</sup> site, Biopile, Enhanced Aerobic and Anaerobic Bioremediation, Reactive Barrier and Phytoremediation were selected for field pilot-tests (ongoing). With regard to phytoremediation, 5 different poplar clones were selected for the 1<sup>st</sup> site (*Populus* spp.), while different herbaceous and arboreal plants were tested in the 2<sup>nd</sup> site with particular attention to oilseed crops for biofuels production. In both sites, morpho-functional plant traits, aerial biomass and root apparatus were monitored, and rhizosphere and bulk soil samples were collected at different depths to perform chemical, agronomic, biomolecular and ecotoxicological analyses. The main evaluations that emerged from these tests are related to the importance to assess the

tolerance of the plants to different levels of hydrocarbons and to the climate site conditions and to verify the efficiency of plants to stimulate the growth of specific microbial strains relevant for plant growth and hydrocarbons degradation. Concerning the 1<sup>st</sup> site, some of the tested poplar clones were able to develop roots that reach even the deeper soil horizon increasing the availability of oxygen and promoting the microbial biodegradation activity; moreover, phytoremediation was also efficient where the level of heavy hydrocarbons concentration was high suggesting the possibility to apply it also for the remediation of the most impacted soils avoiding ex-situ technology. In the 2<sup>nd</sup> site, only few of the tested species (i.e. castor) were able to grow and develop a root system that supported the biodegradation activity of autochthonous microorganisms. In conclusion, the combination of different Nature-based Solutions has shown to be a suitable strategy for the remediation of both sites.

#### **4.01.B Advancements in Bioremediation and Phytoremediation for Addressing Persistent and Emerging Pollutants in Contaminated and Degraded Ecosystems**

##### **4.01.B.T-01 Differential Distribution of Pharmaceutical Compounds in Water, Sediments and Biota of a full-scale Constructed Wetland**

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Pharmaceutically active compounds (PhACs), including antibiotics, are ubiquitous emerging pollutants that pose potential risks for ecosystems and human health. One of the greatest concerns is their effect on the spread of antibiotic resistance, due to the continuous release of these compounds and antibiotic-resistant determinants through wastewater effluents. Constructed wetlands (CW) offer a cost-efficient and eco-friendly solution for the remediation of wastewaters. However, limited studies have evaluated their efficiency in fullscale systems and the accumulation of PhACs in sediments and biota. This study aimed to assess the distribution of 70 PhACs, including 30 antibiotics, through the treatment chain of a full-scale Free Water Surface CW (FWSCW) operating as tertiary treatment of an urban Wastewater Treatment Plant (WWTP). Sampling was conducted in July 2023 in the Empuriabrava CW (ECW, Girona, Spain). Water, sediments, and biota (zooplankton, duckweed and common reed (*Phragmites australis*)) were collected at 5 treatment stages. A set of 70 PhACs, including 30 antibiotics, were analyzed, following extraction, by liquid chromatography coupled to tandem mass spectrometry. Macrolides, quinolones, sulfonamides and trimethoprim were the main antibiotic families detected, at ng/L range. The total sum of antibiotics removal in the combined treatment (WWTP + CW) was 97%, with exception of clindamycin, which increased its concentration during the treatment. Additionally, 17 PhACs from 7 therapeutic families were detected at ng/L, with some compounds decreasing, remaining stable, or even increasing in concentration after treatment (carbamazepine and metoprolol acid). A lower number of PhACs was detected in sediments and biota, at ng/g dried weight level. *P. australis* accumulated  $\beta$ -blockers, psychiatrics and antibiotics, preferentially in roots, even though a few were present in aerial parts. Antibiotics and other PhACs have different removal and distribution behavior along the treatment chain in a FWS-CW. Antibiotics are mostly removed from water after complete treatment, proving the efficiency of CW as tertiary treatment for these compounds. A few of them accumulated in sediments and biota. However, other therapeutic families of PhACs presented positive, zero or even negative removals. They accumulated mostly in *P. australis* roots, which demonstrates the usefulness of this macrophyte for the remediation of PhACs from secondary effluents.

##### **4.01.B.T-02 Reduction of Perfluoroalkyl Acids in Leachate from Industrial Waste Disposal Sites by Bacteria Isolated from Contaminated River Sediments**

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Per- and polyfluoroalkyl substances (PFASs) have been detected in the environment and in living organisms around the world. These PFASs are physically and chemically very stable, due to the C-F bond. They have been widely used in industrial materials because of their water- and oil-repellent properties. However, they can accumulate in high concentrations in humans and wildlife, causing in adverse health effects. Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), which are a type of perfluoroalkyl acids (PFAAs), are known to be persistent, high accumulative, long-range mobile, and toxic. As a result, they still remain in the environment for decades or longer. Bioremediation, which aims to degrade or remove such pollutants by harnessing the biological functions of microorganisms and plants, has attracted attention for the degradation of pollutants. However, there are few reports on PFOS/PFOA-degrading bacteria. The aim of this study is to isolate and identify PFOS/PFOA-degrading bacteria in order to achieve environmental remediation of PFAAs using bacteria. In addition, the selected bacteria will be used to reduce the concentration of PFAAs in actual contaminated water, including leachate from industrial waste disposal sites.

Several bacterial colonies were isolated from PFOS/PFOA supplemented Bushnell Haas Broth (BH) liquid medium incubated with sediments collected from PFOS/PFOA contaminated river sediments. The isolated bacteria were identified by sequencing

analysis of the 16S rRNA gene. PFOS and PFOA concentrations were significantly reduced by 9-31% and 4.7-11%, respectively, by some of the identified bacteria. So far, it is proposed that monofluorinated fatty acids are one of the PFAA metabolites produced by the bacterial consortium isolated from the contaminated sediments. The selected bacteria cultured in the leachate showed a 25% reduction in PFOA concentration.

The isolation and identification of bacteria involved in PFOS/PFOA degradation, and the potential of these bacteria to reduce the concentration of PFAAs in the actual contaminated water, will provide important insights into the application of bioremediation to PFAAs.

#### **4.01.B.T-03 Thinking Out of the Box: *Daphnia* as a Sentinel Species for Environmental Health and Water Reclamation**

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Rapid urbanisation, pollution and climate change have put unprecedented pressure on water resources, culminating in a global water crisis. The sustainable management and reuse of water is paramount for ensuring societal, economic, and environmental well-being. However, persistent chemical pollutants are not efficiently removed by conventional wastewater treatment and prevent the safe reuse of treated water. When wastewater effluent is released into rivers, it eventually finds its way into reservoirs, irrigation systems, and aquifer recharges. These chemical pollutants then enter the human food chain and water supply, detrimentally impacting the health of approximately 92 million individuals annually.

Sustainable technologies that remove persistent chemicals from wastewater are urgently needed. While *Daphnia* have long been recognised as a 'sentinel species', used to identify and set exposure limits on toxic chemicals by regulation, they have not been applied to their full potential. We broaden the use of this sentinel species offering a much-needed advance in the way existing and new risks from chemical pollution are assessed and mitigated. We show how *Daphnia* works as a canary in a coal mine, providing an early warning system of toxicity. And where prevention fails, we develop a *Daphnia*-based scalable, low-cost, low-carbon, and retrofittable technology for tertiary water treatment applications. We demonstrate our technology performance in a first test trial in a real-world environment. We demonstrate bioaccumulation or biotransformation of chemical pollutants and envisage a photocatalytic treatment to break down residual chemicals, enabling the reuse of organic portion of the *Daphnia* sludge.

Our technology could improve the quality of wastewater effluent, meeting current and upcoming regulatory requirements to produce reusable water suitable for irrigation, industrial applications, and household use. By preventing persistent chemicals from entering waterways, we can also prevent environmental pollution. Our circular system promotes the reuse of water and resource for a sustainable future accessible to both developing and developed countries.

#### **4.01.B.T-04 Shifts in the Nitrogen Cycle of a Metal- and Polycyclic Aromatic Hydrocarbon Contaminated Soil Amended with Biochar and Peat**

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Soil is an essential and non-renewable resource, and its human use and ecological functioning is threatened by soil contaminants. Soil amendment with biochar is a sustainable remediation method with a high potential to immobilize many soil contaminants, improve soil health and reduce the need for eternal landfilling. However, it is not known how ecological soil functions, such as nitrogen (N) cycling, in polluted soils benefit from soil amendments of biochar, peat and their combination. Importantly, more field studies are needed that consider the effect of realistic outdoor conditions on pollutant and microbial behavior.

A field trial was established in 2019 with soil contaminated with polycyclic aromatic hydrocarbons (PAH) and multiple metals. The soil was treated with biochar (0, 3 or 6 w/w%) combined with peat (0, 1.5 and 3 w/w%) in a complete factorial design resulting in nine different treatments (n=3). After one growing season analyses were made of grass biomass, soil physicochemical properties, contaminant leaching, plant and earthworm contaminant uptake, and ecotoxicity (acute toxicity and reproduction of earthworms). The focus of our study was to detect effects on soil N cycling using N elemental and stable isotopic ( $\delta^{15}\text{N}$ ) measurements of the system, combined with microbial parameters such basal respiration, microbial biomass and abundances of different N cycling microbial communities.

We showed that peat and biochar additions resulted in a reduced solubility and bioavailability of PAH and heavy metals along with an improvement of soil quality and reduced ecotoxicity. Peat and biochar amendment resulted in an increased microbial

biomass and basal respiratory activity, but a decrease in grass biomass and leaf N content. Changes in the  $\delta^{15}\text{N}$  signatures of leaf, root, and soil indicated that more N was retained in the soil microbial N cycle. Biochar and peat amendments also increased abundances of denitrifying and nitrous oxide reducing organisms, while effect on various nitrifying guilds were less consistent.

Taken together, we demonstrate that soil pollutants and N are immobilized simultaneously, while creating a more favorable environment to microorganisms. Ongoing research at the field trial will demonstrate if the beneficial effects of peat and biochar amendment will remain.

#### **4.01.B.T-05 Effectiveness of the Orion poplar clone in phyto-assisted bioremediation from polychlorinated biphenyl and heavy metals**

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Plant-assisted bioremediation (PABR) is a sustainable technology for recovering contaminated soils. It is based on the synergistic interactions occurring between root systems and microorganisms of the rhizosphere for removing, transforming, and containing hazardous xenobiotics. Poplars were demonstrated to be very effective in favoring the degradation of persistent organic pollutants such as polychlorobiphenyls (PCBs) and inorganic contaminants such as heavy metals (e.g. Zn, Pb, Sn, etc) both in laboratory and field scale applications. This work describes a case-study related to a historically contaminated area, located in Southern Italy, characterized by different levels of PCBs and HMs (medium and high concentrations for both organic and inorganic pollutants). The Monviso poplar clone has been previously planted in 2013 and 2015 in two different areas inside the same site and it was demonstrated to be effective in promoting soil quality restoration, PCB rizodegradation and HM phytostabilization in soils. The present work reports a new PABR performed in a third area still contaminated, using a different poplar clone, the Orion one. The poplar clone was planted in 2022 (about 700 poplar cuttings) for evaluating its capability to promote PCB and HM decrease and compare its adaptability and efficiency with the Monviso clone.

Chemical analysis of soil properties (pH, EC, available P), contaminants (PCBs and HMs), and microbiological analyses (microbial abundance and dehydrogenase activity) were performed before the poplar plantation and at 18 months. Moreover, the structure of the main microbiological groups was assessed by qPCR assays. Pollutant analyses were also performed on biomass tissues (roots and leaves). The main results of the PABR strategy applied with the Orion poplar clone for recovering a historically multi-contaminated area will be described and discussed.

#### **4.01.P Advancements in Bioremediation and Phytoremediation for Addressing Persistent and Emerging Pollutants in Contaminated and Degraded Ecosystems**

##### **4.01.P-Mo316 A QUEST FOR NOVEL FUNGAL SPECIES WITH THE ABILITY TO BIOREMEDIATE POLYETHYLENE IN THE EASTERN REGION OF THE FREE STATE, SOUTH AFRICA: AN ECO-FRIENDLY SOLUTION TO ELIMINATE PLASTIC POLLUTION**

*Nozipho Kheswa, University of Free State, South Africa*

Landfill standards of operation and management are decreasing in South Africa, leading to an accumulation of plastic waste that harms the environment. To address this issue, our study sought to identify fungal species with the potential to bioremediate packaging plastic waste from local landfill sites. We isolated fungal strains from soil samples collected from these sites using an agar medium supplemented with a plastic solution to selectively identify strains with bioremediation properties. Thereafter, plastic strips were inoculated with the isolated fungal strains and the effect of fungal strains was evaluated after 45 days of incubation at room temperature, and plastic bioremediation was evaluated by examining the weight loss of the strips, and changes in plastic chemical composition and intensity using Fourier-transform infrared (FTIR) spectroscopy. Our results identified two promising isolates, SP17MK and SP3MK, that caused significant plastic weight loss of 23% and 33%, respectively. FTIR analysis revealed weaker absorption peaks at 719 and 1,472  $\text{cm}^{-1}$  after fungal treatment compared to the control, and plastic strips treated with SP13INT showed a stretch of the carbonyl group at 1700  $\text{cm}^{-1}$ . South Africa is in dire need to embrace novel approaches to tackle its growing plastic pollution challenges. The present findings could lay the first step towards the use of local fungal species for the bioremediation of plastics.

**Keywords:** Waste management, Landfills, Plastic pollution, Bioremediation, Plastic biodegradation

##### **4.01.P-Mo317 Constructed wetlands as a nature-based solution for removing emerging contaminants**

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Treatment wetlands are among the most efficient and frequently implemented nature-based solutions for wastewater treatment. As for all wastewater treatment systems, there are increasing concerns on how such systems deal with contaminants of emerging concern. While treatment wetlands application for classical pollutants is well described in the literature, data on organic micropollutants is still mostly from laboratory and mesocosm scale studies. The potential to control the emission of pharmaceuticals by such systems is reported. However, works on the performance of full-scale systems over long monitoring cycles are still scarce. Moreover, the fate of antibiotic resistant genes in such systems remains unknown, as existing literature points in different directions (either removal or accumulation). To address these gaps, a full-scale treatment wetland has been monitored for one year to gain knowledge on real-life efficiency and seasonal performance.

The selected treatment wetland is in full operation since 2014, and treats the wastewater from a small village with 100 PE in the Danish countryside. Water and sediment were sampled to analyse organic carbon and nitrogen, microbial community, organic micropollutants (mostly pharmaceuticals and antibiotics), and antibiotic resistance genes.

Results show not only the efficiency of the treatment wetland for nutrient removal from wastewater, but also its capacity to reduce, in general, organic micropollutants. From the overall compounds measured in the system, few antibiotics were sporadically quantified. The removal efficiency for organic micropollutants seems to be influenced by seasonality (lower in winter than autumn, spring and summer), presumably due to the different plant vegetative states during the year. No correlation was found between resistant genes and occurrence of antibiotics. Although antibiotics were not always detected, the *sulI*, *sul2* and *IntI* genes (linked to sulphonamide resistance) were identified in all microbiological samplings and points. Interestingly, the vertical flow bed resulted effective in decrease both emerging contaminants and antibiotic resistance genes. These results can be ascribed to synergic interactions between *P. australis* and its rhizosphere microbiome in the vertical flow sediment.

#### **4.01.P-Mo318 Microbial characterization of a Multicontaminated Marine Sediment in Mar Piccolo Site (Taranto, Italy) and selection of Aerobic and Anaerobic Hydrocarbon-Degrading Bacteria.**

**Bruna Maturro<sup>1</sup>, Maria Letizia Di Franca<sup>2</sup>, Barbara Tonanzi<sup>2</sup>, Carolina Cruz Viggì<sup>2</sup>, Federico Aulenta<sup>2</sup>, Magda Di Leo<sup>2</sup>, Santina Giandomenico<sup>2</sup> and Simona Rossetti<sup>2</sup>,** (1)Water Research Institute (IRSA-CNR), Monterotondo, Italy, (2)IRSA CNR Marine sediments act as a sink for the accumulation of various organic contaminants such as polychlorobiphenyls (PCBs). These contaminants affect the composition and activity of microbial communities, particularly favoring those capable of thriving from their biodegradation and biotransformation under favorable conditions. Hence, contaminated environments represent a valuable biological resource for the exploration and cultivation of microorganisms with bioremediation potential. In this study, we successfully cultivated microbial consortia with the capacity for PCB removal under both aerobic and anaerobic conditions. The source of these consortia was a multicontaminated marine sediment collected from the Mar Piccolo basin in Italy, one of Europe's most heavily polluted sites. High-throughput sequencing was employed to investigate the dynamics of the bacterial community of the marine sediment sample, revealing distinct and divergent selection patterns depending on the imposed redox conditions. Shifts in bacterial communities were observed, shedding light on the adaptation of microorganisms to the tested conditions and their potential roles in PCB transformation processes, but also the biodegradation potentialities of other co-contaminant hydrocarbons such as polycyclic aromatic hydrocarbons (PAHs). From the indigenous microbial community in the highly contaminated sediment, we successfully obtained three distinct enrichment cultures and two isolates. One bacterial consortium showed the potential to reduce the presence of PCBs and PAHs under aerobic conditions (consortium *Ae*), and allowed us to extrapolate two bacterial isolates (*Marinobacter salinus* and *Rhodococcus cerastii*) known to play a role in the biodegradation of hydrocarbons. In addition, two anaerobic cultures were obtained, able to perform PCB biodegradation via reductive dechlorination (consortium *Ana*) and to perform the complete reductive dechlorination of chlorinated ethenes (consortium *Ana\**). Details about the microbial characterizations will be discussed during the presentation. The establishment of consortia with biodegradation and biotransformation capabilities expands the toolkit available for effectively implementing bioremediation strategies, thus enhancing the comprehensive nature-based restoration approach.

#### **4.01.P-Mo319 Investigation of the effectiveness of phytoremediation of dredged sediment contaminated with heavy metals**

**Nina Đukanović<sup>1</sup>, Jelena Beljin<sup>1</sup>, Tijana Zeremski<sup>2</sup>, Mrijana Kragulj Isakovski<sup>3</sup>, Srdjan Roncevic<sup>3</sup>, Nadežda Stojanov<sup>2</sup> and Snežana Maletic<sup>1</sup>,** (1)University of Novi Sad, Serbia, (2)Institute of Field and Vegetable Crops, Novi Sad, (3)University of Novi Sad, Faculty of Sciences, Serbia

Sediment is equally at risk of heavy metal pollution, because the contaminants present in the water system can be absorbed onto the particles, deposited and accumulated in the sediment. In order to remediate soils and sediments contaminated with heavy metals, phytoremediation has attracted great interest from researchers. Given the large number of contaminated sites that cannot currently be used for agricultural production, the goal of our research was to connect the cleaning of polluted sites with phytoremediation and the production of biomass that can be used to obtain clean biofuels. The energy plant rapeseed, which is known for its high phytoaccumulation potential, was sown at the landfill for the purpose of phytoremediation. The rapeseed was sampled in December, April, and June. The samples were subjected to analysis of the total metal content on ICP-MS, and bioaccumulation and translocation factors were calculated. An extremely high yield of biomass was achieved that can be used to obtain biofuel (2.6-2.9 t/ha). The concentration of metal in the energy crop was generally higher in the contaminated soil, compared to the control plot. For Cu, Zn, Pb and Cd, the BAF is approximately at the same level in above-ground and below-



ground biomass, which is consistent with the obtained TF. The high degree of bioaccumulation and translocation of Zn and Cu as essential elements for plants may be due to a higher rate of transpiration to maintain the growth and moisture content of plants. The high BAF and TF of Cd can be attributed to its high bioavailability and physicochemical properties similar to essential micronutrients, which allows it to be easily absorbed and translocated by plants. Pb is toxic in leaves, so sometimes the root prevents its transport to the aerial part and keeps it in the root. The lowest TF observed for Cr is consistent with its lowest mobility and consequently lowest accumulation potential and this is indicated by the higher BAF. We can conclude that an extremely high yield of biomass was achieved that can be used to obtain biofuel. The level of metal uptake and translocation is consistent with the properties and mobility of the metal. However, the choice of plant species is one of the main factors on which the effectiveness of phytoremediation depends.

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#### **4.01.P-Mo320 Ibuprofen-enhanced Biodegradation in Solution and Sewage Sludge by a Mineralizing Microbial Consortium. Shift in Associated Bacterial Communities**

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Ibuprofen (IBP) is a widely used drug of environmental concern as emerging contaminant due to its low elimination rates by wastewater treatment plants (WWTPs), leading to the contamination of the environment, where IBP is introduced mainly from wastewater discharge and sewage sludge used as fertilizer. This study describes the application of a bacterial consortium obtained from sewage sludge acclimated with IBP (consortium C7) and some bacterial strains isolated from C7 to accelerate IBP biodegradation both in solution and sewage sludge. Microbial community changes in the enriched IBP-degrading biomass was investigated using high-throughput sequencing techniques to identify the main bacterial groups potentially involved in IBP bioremoval.

The biodegradation of a wide variety of IBP concentrations in solution by consortium C7 was carried out, reaching 100% IBP degradation in 28 hours for the concentration of 500 mgL<sup>-1</sup>, and 66% IBP was mineralized in 3 days. The toxicity of the solutions towards *Vibrio fischeri* was measured throughout the biodegradation process, being nule when IBP was completely degraded. IBP adsorbed in sewage sludge (10 mg kg<sup>-1</sup>) was quite irreversible (only 12-15%), but after bioaugmentation with C7 a drastic degradation up to 90% IBP was reached in 16 days, with a 5-fold increase in degradation rate.

The bacterial community of consortium C7 was significantly enriched in *Sphingomonas wittichii*, *Bordetella petrii*, *Pseudomonas stutzeri* and *Bosea genosp.* after IBP degradation, with a special increase in abundance of *S. wittichii*, probably the main potential bacterial specie responsible for IBP mineralization. Thirteen bacterial strains were isolated from C7 consortium. All of them degraded IBP in presence of glucose, especially *Labrys neptuniae*. Eight of these bacterial strains (*B. tritici*, *L. neptuniae*, *S. zoogloeoides*, *B. petrii*, *A. denitrificans*, *S. acidaminiphila*, *P. nitroreducens*, *C. flaccumfaciens*) had not been previously described as IBP-degraders. The bacterial community that makes up the indigenous consortium C7 could facilitate IBP bioremediation in contaminated effluents as well as in sewage sludge generated in WWTPs. This is the first time that bioaugmentation with bacterial consortia or isolated bacterial strains have been used for IBP degradation in sewage sludge.

#### **4.01.P-Mo321 Saponin-Enhanced Biodegradation of PAHs from Nonaqueous-Phase Liquids: Towards the Application of Phytogetic Surfactants in Bioremediation**

*Alicia Fernandez-Vazquez, Rosa Posada and Jose Julio Ortega-Calvo, Instituto de Recursos Naturales y Agrobiología, Spain*

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic pollutants often introduced into the subsurface as nonaqueous-phase liquid (NAPL) mixtures, such as creosote or coal tar, that represent long-term sources of risk. The limited bioavailability of these slowly desorbing contaminants must be taken into account when developing nature-based solutions to soil pollution. Saponin is a plant biosurfactant with many advantages, as compared with chemical and microbial surfactants, in terms of sustainability and cost, which has shown a great potential in bioremediation of PAH-polluted soils. The present study seeks to investigate the mechanisms of action of *Quillaja* saponin biosurfactant on biodegradation of <sup>14</sup>C-phenanthrene and <sup>14</sup>C-pyrene with the compounds present either as crystals or dissolved in two NAPLs (heptamethylnonane and creosote).

Radiorespirometry assays with a representative PAH degrader bacterium (*Mycobacterium gilvum* VM552) and with the autochthonous microbial population from a long-term creosote-polluted soil were developed in a biphasic NAPL/water system with a constant interface area. Experiments were carried out with saponin at its critical micelle concentration (CMC, 0.1 g/L) and well above that value (1 g/L). The results showed that saponin, especially above its CMC, promoted the mineralization of crystalline and NAPL-dissolved phenanthrene and pyrene, both with the pure *Mycobacterium* strain and the intact soil microbial population. When present in the NAPLs, the lower chemical activity of the PAHs led to significantly reduced mineralization rates, as compared with the crystalline chemicals, but these rates were enhanced by saponin. Ongoing studies examine the role in this enhancement of the attachment and growth of bacterial cells at the NAPL/water interface, how biosurfactant molecules can interfere in this process, and the balance between bacterial attachment and PAH partitioning into the water phase in the presence of the biosurfactant. Further studies will be carried out to determine if saponin can efficiently reduce end-point PAH concentrations when applied to creosote-polluted soils after conventional bioremediation.

Keywords: bioremediation, pyrene, phenantrene, PAH, biosurfactants, saponin, NAPL.

#### **4.01.P-Mo322 Tactic-Mediated Pore Sealing by Bacteria: Prospecting a New Engineering Component for Risk Reduction**

*José Luis García and Jose Julio Ortega-Calvo, Instituto de Recursos Naturales y Agrobiología, Spain*

The immobilization of organic pollutants, such as PCBs, PAHs and PFAS through sorbents (modified clays, activated carbon) is currently accepted as a remediation strategy for polluted soil and sediment. If the toxic organic chemicals are immobilized, the flux from the soil or sediment to the pore water is low, what results in significant risk reductions. Inspired in this technology, and given the sealing potential of the bacterium *Bacillus subtilis*, already demonstrated for cement bioreparation, we proofed the concept of pore sealing by bacteria as a new bioengineering strategy for reducing the bioavailability of organic pollutants present in microporous media. We employed the strain *B. subtilis* DSM10, a gram-positive, motile bacterium (cell dimensions 2.5 µm x 1.2 µm) with known capabilities for chemotactic mobilization through appropriate chemoeffectors, biofilm formation, and calcium carbonate precipitation. In our study, we employed a strong chemoattractant, gamma-aminobutyric acid (GABA), a chemical signal which is present in many plant root exudates, to trigger the chemotactic response. After 30 min, the bacterial cells were massively attracted into GABA-containing capillaries (1 µL, 200 µm internal diameter), as evidenced by optical microscope observations and quantification through colony forming units inside the capillaries. The incubation in agar plates of the capillaries containing the attracted cells led to bacterial growth and the establishment of cylindrical biofilms inside the capillaries, what was microscopically visible as bacterial plugs formed at the capillary mouth. This process was scaled down to micrometer-sized pores by using membranes with 5 µm and 12 µm pore sizes, through which the tactic response to GABA was tested in bioreactors composed of two chambers separated by the membranes. While the bacterial dispersal through the membranes was severely limited in the GABA-free controls, the GABA gradient generated through the membrane pores caused the massive mobilization of the bacteria through chemotaxis. Similarly to capillaries, GABA-impregnated membranes that were exposed to bacterial suspensions attracted a high number of bacterial cells inside the micropores, which proliferated upon incubation, causing pore clogging. Ongoing studies examine in bioreactor experiments the sealing capacity for organic pollutant transport through the membranes.

#### **4.01.P-Mo323 Enhanced rhizoremediation of a kerosene-contaminated military air-base soil with sunflower and plant-growth promoting bacteria**

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Organic pollutants introduced into the soil by military activities include energetics, chemical warfare agents and other military chemical compounds, generally grouped as potentially toxic compounds (PTC). Contamination by PTC on military bases is mainly caused by spillage of chemicals in storage areas (fuels, oils, lubricants, paints, solvents and corrosives). Their concentrations in the soil of military areas can be unacceptably high and, together with their high toxicity and persistence, can give rise to environmental hazards. Bioremediation promotes the biodegradation of contaminants in soil by stimulating the activity of indigenous microorganisms through the improvement of soil conditions (biostimulation) and through the addition of microbial inocula with specific metabolic characteristics (bioaugmentation). In soils contaminated with organic xenobiotics, it is essential to ensure the dispersion of inoculants over the soil volume and to promote the bioavailability of the contaminants, which in turn will improve the efficiency of bioremediation. The main objective of this study was to design a rhizoremediation strategy for a soil contaminated with kerosene from a military air base (southern Spain), based on the combination of biostimulation and bioaugmentation in the presence of a model plant (sunflower, *Helianthus annuus*). The experiment was carried out under greenhouse conditions using pots with 2 kg of soil. The microbial inoculum used was a mixture of a motile strain (*Pseudomonas putida* G7), which is chemotactic against sunflower root exudates, and a biosurfactant producing strain (*Bacillus subtilis* DSM10). This inoculant was designed to enhance the dispersion of the biosurfactant-producing strain throughout the soil volume by chemotaxis-mediated co-mobilisation together with a mobile strain of *P. putida* G7. Analysis of the soil after treatment showed that kerosene degradation by indigenous microbial communities was more efficient in the presence of plants. Inoculation with *B. subtilis* DSM10 had a negative influence on bioremediation performance, possibly due to potential inhibitory effects of the biosurfactant on native kerosene-degrading microbial communities. However, its co-inoculation with mobile chemotactic strain *P. putida* G7 had beneficial effects on bioremediation performance. Microbial community response to treatments were assessed by 16S rRNA amplicon sequencing and qPCR targeting total and inoculated bacterial communities.

#### **4.01.P-Mo324 Microbiological Investigation of the Combined Addition of Biochar, Bioactivators and Plants on a Soil Contaminated with Petroleum Hydrocarbons**

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Council (IRSA-CNR), Italy

Biochar, a vegetal black carbon produced by the pyrolysis of biomass, is receiving much attention in recent times in soil remediation due to its physicochemical characteristics. Among them, the surface area and adsorption capacity of the pore structure make biochar a suitable substance for toxic compounds immobilization. The use of biochar in synergy with other biological techniques aimed to enhance microbial activity, such as rhizoremediation and bioaugmentation, can represent a powerful strategy to accelerate the removal of organic pollutants from soil. The experiment was designed in order to evaluate the remediation potential towards petroleum hydrocarbons (PHs) from different soil treatments: biochar (SB) and biochar plus bioactivators (SBB), in presence or absence of plants (P) for 9 months. We analysed at different stages the PHs determination and the microbial community structure in terms of the total microbial abundance, composition and diversity. Differences in the microbial community were evident among treatments and time. Interestingly, after 9 months of treatments, the strategy of adding biochar and bioactivators promoted both microbial abundance and specific bacterial groups within the microbial community structure. Moreover, when comparing the biochar plus bioactivators treatment (SBBP) at nine 9 months versus the initial time (SBB) an increase in the number of ASVs (Richness), but a decrease in Simpson and Shannon diversity indexes were observed. Microbial community structure changed from the start to the end of the experiment in all conditions. According to the PCoA analyses, both time and type of treatment (presence of the plant/amendment of biochar/bioactivator addition) shaped microbial community structure and composition. Concurrently, PHs decreased in all treatments and reached a maximum of 70% of removal in the presence of biochar and bioactivators. Preliminary results evidenced that, despite the high PH contamination, the agricultural soil affected by an accidental oil spill maintained an abundant and vital microbial community. The most effective treatment for PHs removal was the concomitant use of biochar and bioactivators. The present study was performed in the framework of BIOCHAR LATIUM Project, CUP J85F21000410002, funded by Lazio Region within POR FESR Lazio 2014-2020 programme.

#### **4.01.P-Mo325 Metatranscriptomics reveal a succession in bacterial communities and functions driving the decontamination of polycyclic aromatic compounds-polluted soils**

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The microbial metabolic pathways and the bacterial populations involved in the degradation of polycyclic aromatic hydrocarbons (PAHs) have been extensively studied. However, most research has been focused on pure cultures or defined consortia in batch experiments. Currently, the challenge lies in exploring the actual *in-situ* processes, considering complex soil microbial networks and encompassing all polycyclic aromatic compounds, extending beyond the 16 regulated PAHs.

A lab-scale biostimulation treatment was conducted on creosote-contaminated soil with nutrient addition (urea and K<sub>2</sub>HPO<sub>4</sub>, C:N:P of 300:10:1) under aerobic conditions for 150 days. Identification and quantification of PAHs, N-PACs, oxy-PAHs were carried out through Soxhlet extraction, followed by fractionation and subsequent analysis using gas chromatography coupled to mass spectrometry (GC-MS). The microbial community dynamics involved in the dissipation of PAHs were analyzed by tracking quantitative (qPCR) and structural (16S rRNA gene metabarcoding) changes at both genomic and transcriptomic levels. Based on recent findings, the potential of native microbial communities to cycle PAH transformation products was evaluated by quantifying Baeyer-Villiger monooxygenases (BVMO).

Transcriptomic analysis of the biostimulated soil revealed a succession in the microbial communities, that extensively removed over 90% of the  $\sum$ 16 PAHs and 80% of the 7 quantified N-PACs. The rapid and nearly complete removal of low molecular weight-PAHs (2- and 3-rings, LMW-PAHs) occurred during the first month and was mainly attributed to members of *Pseudomonas*. Degradation of high molecular weight-PAHs (4- and 5-rings, HMW-PAHs) started afterwards and progressed with slower degradation rates until the end of incubation, with members of *Sphingobium*, *Immundisolibacterales* and *Mycobacterium* identified as major contributors. Expression of PAH-ring hydroxylating dioxygenases (RHD) genes from Gram-negative bacteria showed good correlations with the activity of members of *Pseudomonas* and the removal of LMW-PAHs. RHD gene and transcript copy numbers of Gram-positive bacteria increased during the last month of incubation, correlating with the activity of mycobacteria and the removal of residual fractions of HMW-PAHs. A transient accumulation and subsequent disappearance of various known PAH-metabolites were detected, requiring the participation of specialized native bacteria carrying BVMO to mitigate the associated risk.

#### **4.01.P-Mo326 Investigating the production of sulfonated and hydroxy-sulfonated-PCBs by several organisms through microcosm experiments**

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Sulfonated and hydroxy-sulfonated-PCBs are recently discovered PCB metabolites. Being new chemicals, they are not well characterized yet. Neither information on their fate in the environment nor on their ecotoxicity and toxicity is available. In addition, organisms with the ability to degrade PCBs into sulfonated and hydroxy-sulfonated-PCBs are unknown, such as the metabolic pathway(s) they are involved.

In this work preliminary 3-months microcosm experiments (i.e., flasks) were performed to investigate the production of these metabolites by several organisms: microorganisms isolated from a heavily PCB contaminated soil, from the gut of earthworms (*Eisenia fetida*) collected from the same soil, from leaves of *Quercus ilex* and their associated phyllosphere microorganisms and the *Pleurotus ostreatus* mushroom. Appropriate controls were also set up to investigate photodegradation as well as PCB impurity. 8 PCBs were used as mixture and two of them as single congeners. Metabolites were measured by using an HRMS (i.e., UHPLC-Orbitrap).

PCB metabolites were found in all the flasks but with different levels, and their fingerprint changed depending on the organisms. OH-PCBs was the class for which the highest number of congeners (i.e., different retention time) was produced (53), followed by hydroxy-sulfonated-PCBs (32). Sulfonated-PCBs appeared just 80 days after the start of the experiment. The most efficient system in terms of number and amount of PCB metabolites were leaves of *Quercus ilex* and their associated phyllosphere microorganisms. Even microorganisms deriving from a heavily PCB contaminated soil produced a great number of different congeners. In addition, comparing the experiments, different metabolite congeners were identified in the PCB single congener flasks with respect to the PCB mixture flasks, indicating that probably dechlorination led to the production of other PCB in the mixture flasks that were then or simultaneously sulfonated and/or hydroxylated. This preliminary experiment with different treatments allowed to investigate the most efficient systems in the production of sulfonated and hydroxy-sulfonated PCBs. Moreover, the results represent the first step to hypothesise the metabolic pathway leading to the formation of these metabolites from their parent compounds. However, further experiments will be performed to better understand the environmental fate of these new contaminants.

#### **4.01.P-Mo327 Individual and combined effects of temperature, CO<sub>2</sub>, and soil water content on Summer Rape (*Brassica napus*) potential to remediate Cd contaminated soil**

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Heavy metals are the main group of contaminants contributing to soil pollution, in Europe they constitute up to 35 % of potentially contaminated sites. Phytoremediation is a promising bioremediation technology for the restoration of heavy metal sites by utilizing harvestable plants and posing no adverse effect to soil and environment. Using the energy plant summer rape it is possible to clean the soil while obtaining bioenergy at the same time. The application of phytoremediation is significantly limited by several factors, such as climatic conditions, soil characteristics, rhizosphere microbial community, fertilizer application, the presence of other contaminants. Since phytoremediation is an on-site remediation technology, its efficiency might be also impaired under the ongoing climate change.

This study aimed to analyze the individual and combined effects of soil water content (optimal, reduced, and elevated), temperature, and CO<sub>2</sub> concentration (21/14 °C, 400 ppm CO<sub>2</sub> in the current climate vs. 25/18 °C, 800 ppm CO<sub>2</sub> in the future climate) on the remediation efficiency of Cd-contaminated soil (1-50 mg kg<sup>-1</sup>) for the energy crop summer rape (*Brassica napus* L.). Results showed that summer rape had good resistance to low and moderate Cd soil pollution, as well as the ability to extract Cd from soil, with a significant potential for bioenergy production. Elevated temperature and elevated temperature paired with elevated CO<sub>2</sub> decreased Cd toxicity in *B. napus* by stimulating plant growth, resulting in lower Cd plant tissue concentrations. However, the effect was depending on soil water content (SWC) and Cd soil concentration. Under current climate circumstances, appropriate soil water content resulted in the best Cd removal efficiency with the fewest number of plant harvesting cycles. Future climate conditions enhanced *B. napus* Cd remediation, with the maximum Cd removal rate reported at low and moderate soil Cd contamination (1-10 mg kg<sup>-1</sup>) under higher temperature and SWC. SWC adjustment in response to climate change is critical for boosting phytoremediation capacity since it influences plant biomass development, heavy metal uptake and translocation.

Keywords: *Brassica napus*, cadmium, climate change, phytoremediation, soil water content

#### **4.01.P-Mo328 Bioremediation of Chlorinated Ethenes: Distribution and Abundance of Functional Genes in Situ**

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Chlorinated ethenes (CE), categorized as organochlorines (OCs), globally persist as environmental pollutants, threatening air, soil, and water quality due to toxic metabolites. While conventional treatments are essential, the increasing recognition of natural or substrate-enhanced bacterial degradation of CE and their metabolites is noteworthy. OC biotransformation, primarily through anaerobic reductive dechlorination facilitated by *rdhA* genes (*bvcA*, *vcrA*, *tceA*), involves additional processes like aerobic metabolic degradation. Diverse anaerobic microorganisms, including *Dehalococcoides*, *Dehalobacter*, *Geobacter* and *Dehalogenimonas* utilize OCs in organohalide respiration. Utilizing qPCR, our study assessed the relative abundance of genes associated with total bacterial biomass, oxidative markers (*etnC*, *mmoX*), and reductive markers (*vcrA*, *Dhc*, *Dsb*, *Geo*, *apsA*). In 2022, we introduced nanofibrous biomass carriers into drilled wells at two highly polluted sites in Czechia to capture bacteria with CE degradation capabilities. Through the relocation of nanofibrous carriers from contaminated to non-contaminated aquifers in same sites, our findings shed light on the significant contribution in shaping the genetic landscape of

CE-degrading bacteria. The non-contaminated sites show a low relative abundance of the selected markers compared to contaminated sites. Notably, in both sites, samples from the transferred biomass carriers exhibited an elevated relative abundance of genes, specifically *vcrA* and *Dhc*, compared to levels observed at the low contaminated site. Some bacterial populations rapidly changed, while others maintained a stable profile with slower alterations under varying conditions. The observed increase underscores the potential influence of environmental factors or microbial interactions during the relocation process, warranting further investigation into the mechanisms driving these variations. In summary, our investigation reveals nuanced patterns of chlorinated ethenes-degrading genes, providing valuable insights into their distribution and abundance across diverse environmental conditions, essential for advancing our understanding of microbial bioremediation capabilities.

#### **4.01.P-Mo329 Application of Novel Bacterial Strains for Paracetamol Mineralization in Aqueous and Sewage Sludge Systems**

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Wastewater treatment plants (WWTPs) are identified as significant contributors of Paracetamol (APAP) to the environment. The environmental impact results from the WWTPs inability to fully degrade it. Consequently, the continuous release into the environment, particularly in aquatic ecosystems, is a matter of general concern. Microbial biodegradation is widely recognized as an efficient approach to remove APAP.

This study designed a strategy of bioremediation using bioaugmentation to achieve APAP mineralization in solution and in sewage sludge. *Pseudomonas extremaustralis* CSW01 and *Stutzerimonas stutzeri* CSW02, isolated from sewage sludge by enrichment cultures in the presence of APAP, were able to mineralise around 30% (10 mg L<sup>-1</sup> initial concentration) after 28 days and a DT<sub>50</sub> of 1250 days in the case of CSW01 and for CSW02, a degradation rate of 50% will never be achieved. However, when each of them was inoculated forming two consortia with *Mycolicibacterium aubagnense* HPB1.1 (isolated from a mine sample in the presence of hydroquinone, the main metabolite of APAP) the extent of mineralization significantly increased (up to 58% and 73% for CSW02+HPB1.1 and CSW01+HPB1.1, respectively) and the DT<sub>50</sub> was reduced compared to the treatment by inoculating individual strains at 1 and 9 days for CSW01+HPB1.1 and CSW02+HPB1.1, respectively. After testing the effectiveness of the treatment in the aqueous system, the strategy was extrapolated to a more realistic system (sewage sludge). Similar results were obtained when the bacterial isolates (CSW01 or CSW02) were inoculated individually or together with HPB1.1, achieving an extent of mineralization of around 15% after 28 days (from 50 mg kg<sup>-1</sup> of initial concentration).

#### **4.01.P-Mo330 An Evaluation of the Effluent Quality from The Tshiame Wastewater Treatment Plant (Free State, South Africa), and the Potential Use of Mycofiltration for Improvement in Wastewater Treatment**

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In the eastern Free State, South Africa, there have been reports of high levels of heavy metals in effluent receiving rivers, owing to the release of inadequately treated effluent. Due to infrastructure vandalism at the Tshiame Wastewater Treatment Plant (WWTP), wastewater treatment solely relies on waste stabilisation ponds. Stabilisation ponds are nature-based, and their efficiency could be improved by augmenting with other biological technologies such as Mycofiltration, which is the treatment of contaminated water by passing it through a network of saprophytic fungal mycelium. Mycelia have high biosorption potential for metals. To date, there are no reports on the use of fungi as mycofilters for the remediation of metals from WWTPs.

The present study sought to assess the performance of the Tshiame WWTP pond system, through observing selected physicochemical parameters and heavy metals, and to investigate metal removal by Mycofiltration in both untreated influent and treated effluent.

Physicochemical parameters assessed were pH, Temperature, Electrical Conductivity (EC), Salinity and Total Dissolved Solids (TDS), while the heavy metals were Al, As, Cd, Cr, Cu, Fe, Pb, Mn, Hg, Se, Zn, Mg, Ni, Co, Na, K, Ca, Mo, Ti, P. A mycofilter (made of thatching straw and *Pleurotus ostreatus* mycelia) was cultured for a laboratory small-scale Mycofiltration trial. Mycofilters were packed into pyrex columns (3.3 x 15 cm) and the influent and / or effluent was passed at a constant rate, and the heavy metal content in mycofiltered media assessed. Used mycofilters were characterized using Fourier Transform Infrared Spectrophotometry (FTIR).

Physicochemical parameter values for both influent and effluent were within the recommended guidelines, except for pH which was acidic (pH= 3.7-2.9). The pond system significantly reduced EC and TDS evidenced by lower values in the effluent compared to influent. Levels of Na, K, Mg, P, Fe and Cu were also significantly lower in the effluent compared to the influent, falling within recommended guidelines. While metal analyses of mycofiltered samples is pending, FTIR analysis of mycofilters revealed significant changes in transmittance intensity in the mycofilter used for the influent vs the one used for the

effluent, suggesting greater biosorption of influent. The Tshiamé WWTP stabilisation ponds are fairly efficient. Mycofiltration of influent could potentially further improve wastewater treatment.

#### **4.01.P-Mo331 BIOREMEDIATION AND PHYTOMANAGEMENT STRATEGIES APPLICATION IN SOILS AMENDED WITH SEWAGE SLUDGES**

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During the last decade 2.7-6.5% of the Basque Country surface was considered contaminated. Thus, Basque Country Government developed an inventory of soils that support or had supported potentially polluting activities (Law 4/2015). In this inventory “Landfill 17” located in Gernika-Lumo is included, where sewage sludges from the local WWTP were poured as amendment for decades. In order to decontaminate and recover soil functionality, a combination of bioremediation technologies (involving micro-, phyto- and vermiremediation) was applied. Afterwards, as an additional remediation tool to restore the ecosystem health and diversity a phytomanagement strategy based in natural attenuation and the promotion and maintenance of the native vegetation was carried out *in situ*.

In order to assess the effectiveness of the bioremediation together with the phytomanagement strategy different bioassays including OECD standard toxicity tests or EPA seed tests, were carried out with earthworms and lettuce seeds. To provide a holistic, comprehensive and summarized information about the ecosystem general status, an integrative biomarker response index (IBR/n) was performed. Regarding the results, soil chemical analyses showed a decrease in most of the critical pollutants (Cd, Cr, Ni, Pb, Benzo(a)pyrene) in all the treated plots. Although, microbial parameters did not show a remarkable difference among points, aboveground biological indicators pointed non-phytomanaged plot (MN8) as the area with the worst soil health. Thus, the results obtained indicate that phytomanagement strategies help to eliminate contaminants, help recovering the ecosystem biodiversity and also improve soil health and quality.

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#### **4.01.P-Mo332 MICROTOX Ecotoxicity Test for Assessing Pesticide Soil Toxicity: A Case Study on the Application of Bioremediation Techniques.**

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Trifluralin (TFL) and chlorpyrifos (CLP) are highly persistent pesticides with a strong adsorption capacity on soil particles. Biological strategies are considered as a good option to remediate different environmental compartments. Prior to this study, assisted natural attenuation was used to find the ability of different kinds of soils to biodegrade TFL and CLP. Different specific bacterial strains capable of degrading various pesticides were isolated from soil using the soil enrichment technique. Among the TFL-degrading strains, the most efficient bacterial strain was *Arthrobacter aurescens* CTFL7 (55% degradation of TFL in solution, 10 mg L<sup>-1</sup>), which had not been previously reported in the literature as a TFL-degrading bacterium. For CLP degradation, two CLP-degrading strains, *Bacillus megaterium* CCLP1 and *Bacillus safensis* CCLP2, were isolated from soil and showed the ability to degrade up to 99.1% and 98.9% of CLP in solution (10 mg L<sup>-1</sup>). Different strategies were evaluated to increase the effectiveness of TFL and CLP degradation in soil. These techniques included: i) biostimulation using a nutrient solution; ii) bioaugmentation using a natural microbial consortium, individual bacterial strains isolated from soil, and an artificial bacterial consortium formed by these isolated bacterial strains; iii) bioavailability enhancement using a biodegradable compound, randomly methylated  $\beta$ -cyclodextrin (RAMEB). In this work, ecotoxicity studies were conducted to assess the effectiveness of the studied bioremediation strategies, considering not only their ability to biodegrade a specific pesticide in soil, but also their ability to reduce toxicity, since metabolites can become more toxic than the parent compound. For this purpose, the MICROTOX acute toxicity test was used, which is based on the use of the luminescent bacteria *Vibrio fischeri* as biosensors. In the case of TFL, the ecotoxicity test showed that after application of *A. aurescens* CTFL7 and *A. aurescens* CTFL7 + RAMEB, the TFL-contaminated soil, which initially presented acute toxicity, became non-toxic at the end of the biodegradation experiments. In the case of CLP, it was observed that when the selected degrading strains were individually inoculated into the soil, the toxicity was reduced to undetectable levels.

#### **4.01.P-Mo333 Ecotoxicological assessment of the aqueous soil extracts from post-remediated mining soil in the former Cartagena-La Unión mining district (south-east of the Murcia Province, Spain).**

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Mining activities, particularly those involving metal extraction, pose a significant risk to aquatic systems due to the potential contamination and accumulation of heavy metals. To mitigate these risks, several strategies can be employed to reduce the discharge and accumulation of heavy metals in aquatic environments. These strategies include organic and inorganic amendments addition, as well as remedial actions such as waste management and recycling to minimize the adverse impact on ecosystem and organisms health. One potential organic amendment is biochar, which has been shown to effectively adsorb heavy metals and reduce their leaching into water systems. Composts and manures can be adequate alternatives, as they can enhance soil fertility and promote the uptake of heavy metals by plants, consequently reducing their availability in aquatic ecosystems. Additionally, inorganic amendments such as zeolites and calcium carbonate are also proposed due to their capacity to trap and immobilize heavy metals. The main goal of this work was to evaluate the impact of the range of soil amendments applied to the mining soil on the soil functioning in contaminant and nutrient retention, using direct ecotoxicity assessment with aquatic bioassays. To study this, we produced the aqueous soil extracts, i.e., elutriates, as a proxy to run-off. The elutriates were produced out of the soil sampled from the field plot experiment - non-amended mining soil, and soil amended with different treatments such as compost, biochar, zeolite, calcium carbonate, and their combinations. Freshwater species from different trophic levels – green algae *Raphidocelis subcapitata*, water flea *Daphnia magna* and zebrafish *Danio rerio* were exposed to these elutriates. The evaluated endpoints were algae growth, water flea immobilization and oxidative stress biomarkers, hatching and mutagenicity of zebrafish. Non-amended mining soil elutriate induced toxicity in all the species. The incorporation of amendments substantially decreased the toxicity of the elutriates of the mining soils, with the responses being treatment dependent. Complementary data analysis of the physicochemical characterization (pH, metals, nutrients, etc.) of the elutriates is ongoing, and overall data integration is expected to provide better insight on the adequacy of different treatments and/or their combinations in terms of reducing the pressure from contaminated mining soil on aquatic ecosystem.

#### **4.01.P-Mo334 Field Aging of a Commercial Biochar: Effect on the Retention of Sulfamethoxazole and Ethofumesate Over Time**

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The high mobility of organic pollutants in soils may represent an obstacle to their bioremediation and/or natural attenuation. The addition of adsorbents to soils is an excellent strategy to enhance their retention providing the residence time needed for their degradation by bacteria, fungi, or plants. Biochar (BC), the carbonaceous material produced by pyrolysis of biomass, has been proposed as a soil amendment due to its optimal adsorption properties. However, once in the soil, BCs undergo changes which alter their physicochemical properties and impact their adsorption capacity. Evaluating BC aging under real field conditions is thus crucial to determine its long-term efficiency as an adsorbent. In this work, a commercial BC was aged for one year in the top 0-5 cm of soil plots of an experimental farm located in southern Spain. Unamended and amended soil samples were periodically taken (t = 0, 1, 3, 6, and 12 months) and their retention capacity for the antibiotic sulfamethoxazole and the pesticide ethofumesate was assessed by batch adsorption and leaching experiments. Changes in the physicochemical properties of the BC particles upon aging were evaluated by determining their elemental composition and registering their Fourier-transform infrared spectra and scanning electron micrographs. The results showed that the addition of the BC to the soil greatly increased the adsorption of both compounds compared to unamended soil. However, the adsorption capacity of the adsorbent decreased gradually over time, affecting leaching rates. The characterization of the adsorbent indicated that aging mainly consisted of physical changes at the BC particles' surface. Despite the loss of adsorption capacity of BC over time, one-year aged BC-amended soil samples retained the pollutants to a much greater extent than unamended soil samples. This means that, after one year of residence in the soil, the BC could continue retaining much of the investigated pollutants, which would favor the restoration of the contaminated soil through suitable bioremediation processes. Acknowledgment: Financed by project P20-00746 of Junta de Andalucía (Spain) and projects PID2020-112563RB-I00 and PID2022-137187OB-I00 of the Spanish Ministry of Science and Innovation, with (EU) FEDER funds.

#### **4.01.P-Mo335 Evaluating the Significance of Two Different Biochar Dissipation Pathways in Soil**

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Biochar, as a product derived from the pyrolytic conversion of biomass, can perform several ecosystem functions, two of which are particularly important: long-term soil quality improvement and carbon sequestration. The degree to which these benefits will be realized is largely determined by the stability of the biochar within the soil. Despite generally being considered highly persistent, a mild depletion of biochar stocks, specifically the Pyrogenic C fraction, was observed during a 12-year field experiment set up in Donndorf/Eckersdorf, Germany. Hence, the question arises: can the identified loss over this relatively short time period be attributed to some transformation of biochar, or is it simply the result of its physical transport. To address this question, samples collected from the Donndorf/Eckersdorf trial were analyzed for the amounts of free benzene polycarboxylic acids (BPCAs), as they serve as molecular markers for the degradation of Pyrogenic C. The samples were obtained from two experimental variants: one with the individual application of a high dose of biochar (31.5 t/h), and the other combining biochar (31.5 t/h) with composted material (70 t/h). Sampling was conducted in 2009, prior to the experiment's establishment, and subsequently at two-year intervals in 2013 and 2021. In 2009 and 2013, samples were taken at depths of 0–

10 cm and 10–30 cm, while in 2021, samples were collected at a depth of 0–30 cm. The soil pH ranged from 5.4 to 5.8, and the texture was as follows: 62% sand, 12% silt, and 26% clay. The biochar was produced through the pyrolysis of beech and pine wood chips. The analysis of free BPCAs was conducted using GC-FID. In most of the analyzed soil samples free BPCAs were not detected. In the remaining cases, free BPCAs were identified, at both investigated soil depths, but only in amounts that can be considered relatively low, ranging from  $3.96 \times 10^{-5}$  to  $5.51 \times 10^{-2}$  g/kg. Given that the pH and sandy loam texture of the examined soil did not favor the loss (further oxidation or leaching) of free BPCAs, their absence or low abundance can be considered a clear indication of the high stability of the applied biochar. Therefore, the observed loss of biochar should be attributed to its physical transport. However, as absence or low levels of free BPCAs may also result from the high detection limit of the GC-FID method, to confirm the obtained results, samples will be further analyzed using the LC-VWD.

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#### **4.01.P-Mo336 Effectiveness of a hydrocarbon contaminated soil bioremediation process**

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Bioremediation of total petroleum hydrocarbons soil (TPH) can be achieved through various techniques. Bioaugmentation upon injection of selected functional microbial strains, among others, enables immediate degradation. The use of microorganisms that produce surface-active compounds enhances solubility, mobility, bioavailability, and subsequent biodegradation of hydrophobic organic compounds by emulsifying or solubilizing the hydrocarbons.

The evaluation of soil toxicity becomes an additional but fundamental step of the bioremediation process, being an indicator of the success of the biological treatment. In fact, the effectiveness of the bioremediation process cannot be evaluated only through analytical monitoring, which is complex due mainly to the characteristics of the matrix.

In the framework of Italian PNRR project “Return”, the goal of this work was to determine the effectiveness of a microbial formula in soil TPH bioremediation by pollutant biodegradability assessment and the toxic effects attenuation with an approach that involves chemical, microbiological, respirometry and ecotoxicological evaluation.

This work started with the selection of microorganisms for biosurfactant production using 50 aerobic bacterial strains from the ENEA-MIRRI Microbial Collection. The strains were grown on selective liquid media (BDH) and the surfactant production was followed up to 10 days using Oil Spreading Assay and E24 assay on the supernatants, to test their capacity to produce biosurfactant. The best biosurfactant producers, (i.e. A2-5 *Pseudomonas glycinis*, OSS19 *Rhodococcus qingshengi*, SM41, *Kokuria polaris*, SME 2.4 *Arthrobacter humicola*, SME 2.18 *Microbacterium hydrocarbonoxydans*) were further investigated. Cross-streak tests among strains were performed to check the compatible strains to be pooled to set-up the formula for bioaugmentation in biometer flasks.

Ecotoxicological tests were carried out on either the solid fraction and aqueous and organic extract of TPH soil at different bioremediation stages. Root elongation toxicity test with *Lepidium sativum*, *Sinapis alba* and *Sorgum saccharatum* assessed toxic effect directly in soil fraction. The bacteria *Aliivibrio fischeri* and the crustacean *Daphnia magna* were used to assess the acute toxicity of leachates while a chronic test was performed with the algae *Raphidocelis subcapitata*. The combined use of these test organisms proved to be a viable tool for demonstrating the recovery of soil quality and health.

#### **4.01.P-Mo337 Amplicon Sequencing Reveals Active Functionalities in Petroleum-Degrading Microbiome**

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Bioremediation of a heavily polluted soil with varying fractions of hydrocarbons ranging from n-C8 to n-C40 was investigated. Total petroleum hydrocarbons (TPH) concentrations were 490,631 mg kg<sup>-1</sup> in the surface (0 – 15 cm), 320,971.60 mg kg<sup>-1</sup> at 1 m depth and 81, 434.86 mg kg<sup>-1</sup> at 1.5 m depth. Remediation of the oil-polluted site began with site mapping where approximately 2 plots of land (Length = 120; Width = 125) were cordoned off from the massively polluted expanse of land. Prior to remediation proper, samples were taken from different points of the mapped site (4°47'41.1"N 6°51'47.2"E - 4°47'42.4"N 6°51'46.4"E) representing the surface soil (0 – 15cm) and subsurface soil (30cm – 1m and 1m – 2m depth). Unpolluted soil samples were also collected approximately 1000 meters away from the artisanal refining site (4°47'26.3"N 6°53'32.1"E) to serve as reference soil for comparison. Remediation involved making, breaking and re-making of ridges at 2 weeks interval to improve aeration and proper mixing of all components. Microbiome analysis was done using 16S rRNA amplicon sequencing on Illumina MiSeq platform. To determine the core bacteria across the different operational taxonomic units (OTUs) in all polluted samples, only OTUs with at least 20% abundance were screened and selected. At least 7 of the core bacterial classes were found to have a minimum prevalence of 70% and included *Alphaproteobacteria*, *Betaproteobacteria*, *Actinobacteria*, *Acidobacteriia*, *Anaerolineae*, *Gammaproteobacteria* and *Bacilli*. At the family level the core bacteria with minimum prevalence of 70% included *Acetobacteraceae*, *Koribacteraceae*, *Bradyrhizobium*, *Anaerolinaceae*, *Hyphomicrobiaceae*, *Commamonadaceae*, *Burkholderiaceae*, *Alcaligenaceae*, *Sphingomonadaceae*, *Xanthomonadaceae* and *Bacillaceae*. Further investigation of the core microbiome during remediation revealed six bacterial genera had at least 70%



prevalence across the samples and included *Anaerolinea*, *Candidatus Koribacter*, *Acidocella*, *Burkholderia*, *Bacillus* and *Parvibaculum*. High-throughput sequencing and bioinformatics revealed signature biomarkers (for pollutant degradation and biosynthesis), functionalities and keystone bacterial phyla involved in field-scale remediation and site recovery. These high-end techniques unequivocally established ecosystem and biodiversity restoration thereby promoting citizen science, indigenous knowledge, UN SDGs 4, 12, 13, 15 and 17.

#### **4.01.P-Mo338 Phytoremediation: a tool to remove chemically distinct antimicrobial resistance drivers?**

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The environment is increasingly being recognized for the role it might play in the global spread of clinically relevant antimicrobial resistance (AMR). Evidence is emerging that in addition to antibiotics, biocides and heavy metals also contribute to the growing problem of AMR. Following release into the aquatic systems (e.g. rivers, lakes), these contaminants may contribute to the dissemination of AMR by enriching resistance gene determinants via co-selection mechanisms. There is an urgent need to develop tools and processes to remove these contaminants from the environment to challenge the spread of antimicrobial resistance. Aquatic phytoremediation is a recently adopted methodology to remediate contaminated matrices via sequestration and enhanced degradation of chemical pollutants in aquatic environments. However, to date our understanding of the potential role phytoremediation can play in contaminant removal has largely focused on contaminants from particular chemical classes and rarely considers true environmental complexity. A common aquatic hyper-accumulator plant, *Lemna minor*, was selected to assess the potential for phytoremediation of common AMR drivers (metals, antibiotics, biocides), individually and together as a co-contaminant mixture. Under controlled laboratory exposure two metals (cadmium (Cd) and chromium (Cr), two antibiotics (trimethoprim and ciprofloxacin), and two biocides (diazinon and atrazine) were used to assess the potential of co-contaminant competition on uptake and accumulation by *Lemna* sp over four days. Contaminants were spiked into the exposure media at 150 ng/L. At the end of the exposure media was sampled for analysis and plant material was harvested and extracted using a validated method (0.1M Na<sub>2</sub>EDTA McIlvaine buffer and 2mM phosphoric acid in acetonitrile) and solid phase extraction clean up step. Quantification of analytes of interest was achieved using LC-MS/MS. Mitigation measures to combat AMR are needed which account for chemical complexity in the natural environment and the fact that exposure does not occur in isolation to a single driver.

#### **4.01.P-Mo339 Photocatalytic Transformation of PFOS**

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals widely used in industrial and consumer products for their water- and grease-resistant properties. They are known for their persistence in the environment and resistance to degradation, and adverse health effects. Perfluorooctane sulfonate (PFOS), C<sub>8</sub>-PFAS, was one of the most widely applied PFAS, due to low surface tension and great surfactant properties. Although PFOS use is now restricted, the past applications resulted in widespread contamination, posing a challenge in managing their impact on ecosystems and human health. The process of photochemical degradation has demonstrated itself as an economical, effective, and environmentally sustainable method for the degradation of various compounds. Processes employing titanium dioxide (TiO<sub>2</sub>) and aluminum oxide (Al<sub>2</sub>O<sub>3</sub>) have gained attention as a promising avenue for the photocatalytic degradation of PFAS.

In this study, eight different Ti and Al based catalyst were employed for the investigation of photocatalytic PFOS degradation. The maximum PFOS concentration used was 50 ppm. The experiments were conducted at 20 °C in an open cylindrical polypropylene reactor, and a simulated solar radiation lamp (Solimed BH Quarzlampe) was used as a light source. Photocatalysis was monitored up to 8 hours. The range of degradation was 50-78%. Analysis of degradation products indicated that the dominant method of transformation is oxidation. Additionally, ecotoxicity to *Allivibrio fischeri* of both PFOS and photocatalytic degradation products was assessed. The obtained results confirmed the possibility of remediation of PFOS and related compounds in aquatic environment. Further studies will focus on the optimization of this approach and elucidation of the complete transformation mechanism.

#### **4.02 Biodiversity, Ecosystem Services and Ecological Risk Assessment: Advances and Challenges**

##### **4.02.T-01 Navigating Management Strategies in Mediterranean Coastal Wetlands: An Ecosystem Services Perspective**

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Mediterranean coastal wetlands provide a wide range of ecosystem services. However, their ability to deliver these services can be disrupted by human activities, impacting both the ecosystems and the benefits society obtains from them. Research on eutrophication's impact on wetlands has been thorough, but the wider effects of agricultural chemicals on ecosystem dynamics are less explored, with traditional toxicity tests focusing on individual species rather than whole ecosystems. The AQUATOX model overcomes this limitation by simulating the combined effects of various stressors on ecosystem structure and functions, aiding in more effective ecosystem conservation efforts. In this study, we used the AQUATOX model to assess the effects of nutrient and pesticide inflows in Mediterranean coastal wetlands, using the Albufera lake (Valencia, Spain) as a case-study.

We quantified trade-offs between ecosystem services under various management scenarios, identified critical thresholds for nutrient and pesticide levels, and formulated sustainable management recommendations. To do so, we entered meteorological and hydrological data into our model and created a time series for nutrient and pesticide inflows based on this data and existing research. We integrated biotic community data, established trophic links from literature, and after calibrating with phytoplankton and zooplankton data, we used the model to simulate various management scenarios, including different nutrient and pesticide inputs reflective of agricultural practices. Then, we quantified Albufera Lake's ecosystem services in monetary terms, covering provisioning services like hunting and fishing, calculated from game bird abundance and eel biomass, and recreational services such as recreational fishing, birding and tourism based on carp biomass, bird abundance, and water clarity. We also assessed three regulation services—carbon sequestration and water depuration of nitrogen and phosphorus—estimating their economic value through market prices and cost savings in water treatment. By applying this workflow, we provide a holistic understanding of the necessary trade-offs between agricultural use, biodiversity conservation, and ecosystem service provision in Mediterranean coastal wetlands.

#### **4.02.T-02 Accounting for the Impact of PPPs on Ecosystem Services by Ecological Models: An Example for Pest Control**

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In the currently ongoing, EFSA-funded AENEAS project, a protocol for evaluating the impact of plant protection products (PPPs) on the ecosystem functions that provide ecosystem services is developed. The protocol provides a step-by-step approach for linking effects on vulnerable taxa to ecosystem function delivery within agricultural landscapes. Population models or additionally field studies are proposed in this protocol to assess the impact of PPP exposure on the population dynamics of vulnerable taxa within agricultural landscapes. This contribution provides an example on how population models can be utilized to simulate pesticide impacts on ecosystem services delivered by non-target arthropod species. We illustrate this by using *Erigone atra* as an example species, based on an implementation for *E. atra* in the ALMaSS framework to simulate population abundances with and without pesticide application in an agricultural landscape. Simulated population abundances were translated into the potential for pest control by using the concept of ecological functional responses.

Resulting *Erigone* abundances extracted from ALMaSS for the whole landscape window, showed that 90% (and 99.9%) mortality led to an average reduction of abundances of about 20% in the summer months. The potential ecosystem function of pest control was calculated based on the specific functional response and was found to be decreased by stronger pesticide effects at population levels. When aphid numbers are lower, the potential for the ecosystem function is also lower.

Analyses of derived NORs of the population abundances with and without the impact of pesticides come close to the conceptual ideas as outlined in the EFSA scientific opinion on recovery. Simulated population abundances can be connected via functional relations to ecosystem functions, so that a quantitative link from population effects of a pesticide to impacts on ecosystem functions can be calculated. Landscape-level assessments consider subpopulation impacts, metapopulation dynamics, and landscape heterogeneity, providing a comprehensive assessment of PPP impacts on ecosystem function (i.e. potential ecosystem service delivery). Data scarcity points towards the need for models for the support of the calculation of NORs and the impacts of pesticides on ES delivery. The calculation of model-derived NORs as demonstrated for *E. atra* in this study appeared absolutely feasible.

#### **4.02.T-03 Individual effects to Ecosystem Services: Insights from DEB IBMs on Freshwater Shredder Species**

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An ecosystem services approach to environmental risk assessment aims to understand how chemicals impact the ecological processes and functions that underpin the delivery of ecosystem services. Ecological functions are performed by populations of species. While toxicity tests often rely on single-species endpoints, an ecosystem services approach requires consideration of population responses – which can be influenced by intraspecific interactions. Mechanistic effect models, like Dynamic Energy Budget Individual-Based Models (DEB IBMs), offer a bridge from individual-level effects to population-level responses, accounting for ecological complexity and changes in population structure and activity – important for monitoring ecological functions.

In freshwater systems, invertebrate populations, particularly shredders, play a crucial role in ecological functions. This study focuses on the energy dynamics of two freshwater shredder species, *Gammarus pulex* and *Asellus aquaticus*, linking individual responses to the performance of ecosystem services: provisioning (i.e., food) and cultural (i.e., angling) services provided by fish and regulation and maintenance services related to nutrient cycling and leaf decomposition. We explore both direct and indirect effect scenarios, including reductions in feeding rate, assimilation efficiency, and resource availability and resource quality (from 10 to 100% effect level).

Utilising DEB IBMs, we simulated populations of the two shredders for 20 years. Treatments were applied at year 10 following the initial model stabilisation period. The 95th percentile values of adult shredder biomass and population

consumption rate of the 100 replicates were tracked over 10 years. The findings highlight the differential sensitivity of mechanisms studied. Reductions in assimilation efficiency, food quality, and quantity significantly impacted adult biomass, emphasising their pivotal role in shaping population dynamics and potential effects on food for fish. Diminished resource availability led to reduced leaf processing power in both species. In conclusion, both direct and indirect effects on energy uptake by shredders influenced ecological functions, that underpin the delivery of ecosystem services. DEB IBMs can provide a valuable tool for understanding the intricate relationships between individual responses and ecosystem services, facilitating informed decision-making for aquatic ecosystem management.

#### **4.02.T-04 Evidence of Chemical Impact on Biodiversity**

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Although the European Green Deal and in particular the Zero Pollution Action Plan, the Chemical Strategy for Sustainability (CSS), and the Biodiversity Strategy for 2030 acknowledge pollution negative impact on biodiversity, still this threat seems to be misestimated because of a lack of a crystallized body of evidence that establish causality between chemical exposure and biodiversity. Identifying studies which extrapolate eco-toxicological endpoints measured at the (sub)individual level to assess the effects on diverse biodiversity measures would provide the basis to build such evidence. To this purpose, we are performing a review study based on a PEO framework (Population/Exposure/Outcome). In particular, population included the biota observed and tested within an ecotoxicological study, exposure included any exposure to defined chemical(s) and concentration(s), and outcome of interest was defined as any biodiversity measure (e.g. loss of number of individuals in a wild population, loss of a functional trait) or toxicological endpoint(s) likely to negatively affect biodiversity (e.g. reproductive endpoints). Twenty-five thousand peer-reviewed publications were obtained from Scopus and Web of science Core Collection databases. Using predefined inclusion/exclusion criteria, all articles are screened first on titles and abstracts, then on full-text. This step is currently ongoing. Afterwards, the data extraction will mainly focus on the following key information: type of chemical(s), type of study (lab, semi-field, field and single, multiple species), biological organization level and toxicological endpoint(s) tested, method of extrapolation to higher biological organization levels, inclusion of any species interaction. Data analysis of the extracted data will highlight the main classes of chemicals and taxa for which evidence of impact of exposure on biodiversity exists, and the main methods that have been used to generate such link. The outcome of the analysis will be presented at the conference.

#### **4.02.T-05 Assessing Risks to Biodiversity from Exposure to Chemicals: Findings of an ECETOC Task Force on Biodiversity Definitions, Metrics, and Methodologies**

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In accordance with global efforts to reduce the negative impacts of chemicals on biodiversity, the EU Commission has set ambitious goals for mitigating the entry and effects of chemicals in the environment. However, these goals are hindered by unclear definitions, metrics, and relationships of biodiversity with chemical regulation. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) initiated a Task Force to address some of the challenges. Here we report on the outcomes from the Task Force subgroup overviewing biodiversity definitions and metrics, as well as methods for assessing and quantifying biodiversity. We surveyed the peer-reviewed literature, with a primary focus on reviews, perspectives, and meta-analyses. Preliminary results revealed a wide array of both definitions and metrics for assessing biodiversity. Definitions ranged from descriptive to quantitative and probabilistic. Metrics either focused on a single measurement or integrated multiple measurements into a single index. Proxies for biodiversity (e.g., provision of ecosystem services) were also identified as metrics. The majority of metrics were associated with taxonomic diversity, whereas functional diversity received comparatively less – although increasing attention. More dynamic metrics included temporal and spatial turnover instead of focusing on snapshots of community composition. Metrics varied in their scalability from local to global assessment, and also varied in their ability to be normalized for use across different sectors and applications. As the world moves towards unifying goals of environmental stewardship, emerging frameworks (e.g., The Global Biodiversity Framework, The Essential Biodiversity Variables) are helping to consolidate these definitions and metrics. This will be vital to ensuring that chemical regulations prescribe risk assessment approaches that fully align with those goals.

#### **4.02.P Biodiversity, Ecosystem Services and Ecological Risk Assessment: Advances and Challenges**

##### **4.02.P-Th438 Modeling Chemical Source-to-Damage Pathways in Terrestrial Ecosystems**

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Understanding the intricate relationship between chemical exposure and its multifaceted impacts within terrestrial ecosystems is pivotal for effective terrestrial biodiversity conservation and chemical regulation. However, we still lack consistent methods to quantify various chemical pollution damages within terrestrial ecosystems. To address this gap, we introduce a systematic approach to model chemical source-to-damage pathways and their repercussions on various dimensions of terrestrial ecosystems. First, we focus on modeling chemicals' fate and ecological exposure using geospatial multimedia models. These models aid in unraveling the complex fate of chemicals across environmental media and their propagation in terrestrial species networks. Second, ecological exposure is combined with organism-level effects to derive ecotoxicity effects. These are then linked to species loss by mapping ecotoxicity pressure to species abundance and richness patterns. Third, we model damage on genetic diversity going beyond species loss by merging multi-level data linking chemical activity with adverse phenotypic outcomes within and across species, where genetic targets for chemicals serve as fundamental markers in this exploration. Fourth, we model damage on functional diversity by establishing relationships between species-specific ecotoxicity data, species abundances, and functional traits to inform the derivation of metrics representing functional diversity loss across varying chemical concentrations. Finally, we link the aforementioned metrics (species and functional diversity) to damage on terrestrial ecosystem services, exploring their interconnectivity and quantitatively modeling the propagation of chemical effects across trophic levels and their subsequent damage on relevant services provided. Model results are spatialized and scaled up to cover wider geographical regions and evaluated against existing and new monitoring data along the modeled source-to-damage pathway. Our systematic approach offers a comprehensive understanding of chemical exposure's complexities and its profound implications for terrestrial biodiversity, aiming at providing valuable insights for more efficient environmental assessment of chemicals.

Keywords: ecotoxicity assessment; species loss; genetic diversity, functional diversity, ecosystem services

#### **4.02.P-Th439 Scaling Up: An Ecosystem Services Approach To Assess Chemical Risk To Recreational Fisheries Within A River Network**

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Freshwater recreational fisheries are an important cultural ecosystem service enjoyed by millions of anglers globally but are threatened by multiple stressors, including chemical pollutants. Here we investigate how an ecosystem services approach can be applied to assess the risk of chemicals to recreational fisheries and explore how this assessment varies depending on whether it is performed at the river reach or the river network scale. Our study is focused on trout fisheries and uses a spatially explicit individual-based ecological model (InSTREAM v 7.3), adapted to consider toxic effects, combined with an evaluation of angler satisfaction. The model was applied to a simulated river network of 20 reaches and three stream orders. To investigate the influence of toxic chemicals at the landscape scale, ten reaches were randomly selected where common effect endpoints (growth rate, fecundity, and survival) in trout individuals were inhibited. Varying levels of inhibition (low, medium, and high) were simulated and outputs of angling stock abundance and location within the network were analysed. Outputs were compared to results for single reaches simulated independently. Results show that the responses of the angling stock to chemical stress vary depending on stream order, reach location, and the endpoint inhibited. Results also show that patterns identified on a reach scale are not simply mirrored at the network level as chemical stress effects are mitigated from the point of exposure when part of a metapopulation. Implications for angler satisfaction and ecological risk assessment will be discussed. This study illustrates the potential complexity of scaling ecosystem service assessment risk assessment from local to landscape scale.

#### **4.02.P-Th440 Understanding the Impact of Anthropogenic Environmental Change on Freshwater Biodiversity across Spatiotemporal Scales**

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Threats to biodiversity, such as land use change, chemical pollution and climate change, occur over multiple spatial and temporal scales. To understand the future impact of different climate and pollution scenarios, we must study the combined impact of these threats and, where possible, establish a baseline of pre-impact biodiversity. Here we put forward and demonstrate the use of a holistic biodiversity monitoring method, combined with machine learning analysis, to identify the effects of anthropogenic environmental change.

Our work focuses on lakes as they are key in delivering numerous ecosystem functions and services and they act as receivers of pollution to the landscape. By using multi-marker metabarcoding of environmental DNA, we are able to monitor the whole community, over time (using lake sediment cores) or through space (using multiple contemporaneous lake samples). We find that combinations of stress (eg insecticide pollution and extremes of temperature) have the greatest impact on biodiversity, and

that traditional monitoring metrics do not adequately capture the dynamics of the community as a whole. We present and demonstrate the use of machine learning approaches to identify and prioritise the most impactful environmental changes.

#### **4.02.P-Th441 Linking ecological and ecotoxicological risk to ecosystem services degradation of Alento catchment (Southern Italy)**

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Assessing and managing risks of anthropogenic activities to ecological systems is necessary to ensure sustained delivery of ecosystem services (ES) for future generations. Aquatic ecosystems provide essential benefits to our global society and human wellbeing. Food, drinking water, cultural services, and recreational fishing are among the obvious ES provided by river catchment.

However, river catchment is continually threatened by human activities, which interferes with species diversity as well as ecosystem function, both of which are essential for sustaining the environment.

The Cilento region hosts a National Park and exhibits a territorial context with a combination of cottage industrial, domestic/touristic, and agricultural activities. Consequently, organic, inorganic, and emerging contaminants are released into aquatic environment, posing serious threat due to their hazardous characteristics. This area represents an interesting case study due to its integrated system of storage dams, which accumulates stream flow rates throughout the year. The water is then supplied to the Vallo della Lucania network, supporting irrigation, drinking, energy production, recreational activities, and maintaining high biodiversity. GIS analysis was used to integrate available territorial and environmental data to select sampling areas to be seasonally monitored. Water matrix was characterized by chemical analyses, and by a battery of ecotoxicological tests with organisms from different trophic levels (bacteria, algae and crustaceans).

The propose of this study is to assess how to integrate the risk posed by chemicals on Alento catchment with the measured ecotoxicological effects in the view of ES protection. In fact, among the advantages of using an ES approach for ecological risk assessment (ERA) the increased integration of the risk deriving from multiple stressors and multiple environmental compartments is particularly relevant and allows a more effective communication by highlighting the direct and indirect benefits stakeholders.

#### **4.02.P-Th442 Enhancing Government Processes and Communication Through Bayesian Networks**

*April D Reed and Wayne G. Landis, Western Washington University*

This research focused on creating and communicating a Bayesian Network (BN) decision support tool designed for application in United States government processes with a specific focus on the Natural Resource Damage Assessment and Restoration (NRDAR) program. Stakeholders from diverse backgrounds contributed to the tool's development, addressing the intersection of environmental science, law, and economics. A case study was conducted on the fish resources of the Little Mississinewa River, a PCB-contaminated Superfund site in mid-eastern Indiana and the larger Mississinewa River, to ensure comprehensive coverage of potentially injured riverine habitat. For the case study, we developed a BN as a framework to evaluate the connections between chemicals released at the study site and the resulting injury to fish species. The resulting BN not only provides utility to government agencies as an injury assessment tool, but also facilitates effective science communication. The graphical nature of BNs simplifies visualization of complex relationships through graphical representation. Nodes and arrows represent variables and causal relationships making it accessible for experts and non-experts alike. Bayesian networks explain not just associations but also the cause-effect relationships within a system which is crucial for decision support in programs like NRDAR. Bayesian networks inherently convey uncertainty for more nuanced and realistic interpretation of data. Bayesian networks can be adapted for varying levels of expertise presenting detailed analyses for experts and simplified visualization for non-experts. Bayesian networks can act as a common language, facilitating collaboration among experts, ensuring a unified model that incorporates the knowledge and needs of all stakeholders. Using Bayesian networks for government processes and programs such as NRDAR not only provides transparent support and communication of decision-maker choices and the associated uncertainties, but also enhances science communication by offering a versatile method for representing, analyzing, and conveying the complex and interconnected relationships of ecosystems in a way that is accessible to diverse audiences, including government agencies.

#### **4.02.P-Th443 Assessing risks to biodiversity from exposure to chemicals: findings of an ECETOC Task Force on the regulatory context**

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In accordance with global efforts to reduce the negative impacts of chemicals on biodiversity, the EU Commission has set ambitious goals for mitigating the entry and effects of chemicals in the environment. However, these goals are hindered by unclear definitions, metrics, and relationships of biodiversity with chemical regulation. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) initiated a Task Force to address some of the challenges. Here we report on the outcomes from the Task Force subgroup focussing on EU chemical regulation and strategic documents, that looked into regulatory context for considering biodiversity in chemical risk assessments. We mapped EU legislative documents and overarching strategies concerning biodiversity conservation and/or regulation of chemicals, as well as selected global initiatives influential at EU level. A deep dive into the most relevant documents analysed i.a. how biodiversity metrics, protection goals and impact assessments are defined and how all would link into the EU's political ambitions and strategies. Preliminary results revealed a broad definition of biodiversity requiring further specification for implementation. Likewise, protection goals are usually provided on a rather general level. Metrics vary based on definition and specification level applied in the regulatory documents, as well as on the scope and purpose of the regulatory documents and the expected site of chemical action in the environment. Biodiversity is indirectly addressed via protection of surrogate groups or functions. The further evaluation of the EU Biodiversity Strategy in relation to chemical regulation is currently ongoing. The aim is to identify how existing chemical regulations may align with the EU Biodiversity Strategy, if quantitative links can be established and how these may expand on the existing regulations. The findings provide a starting point for a multi-stakeholder discussion aiming to advance the chemical regulations with respect to biodiversity impacts. The outcome of the work of this ECETOC Task Force will be a short overview/perspectives paper and a workshop involving relevant stakeholders for EU regulations.

#### **4.02.P-Th444 Assessing Risks to Biodiversity from Exposure to Chemicals: Findings of an ECETOC Task Force on Current and Future Research Directions**

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In accordance with global efforts to reduce the negative impacts of chemicals on biodiversity, the EU Commission has set ambitious goals for mitigating the entry and effects of chemicals in the environment. However, these goals are hindered by unclear definitions, metrics, and relationships of biodiversity with chemical regulation. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) initiated a Task Force to address some of the listed challenges. Here we report on the outcomes from the Task Force subgroup that overviewed EU-commissioned research projects to better understand how new knowledge can be leveraged to inform regulatory frameworks as well as to identify research gaps that could become the scope of future funding calls. Preliminary findings indicated that only recently EU Horizon calls start to focus on research questions related to biodiversity and measures to halt the decline, thus the acknowledgement of their respective research findings in shaping chemical regulation is yet to come. However, while the EU Biodiversity Strategy and the relation to chemical regulation is focusing on a defined regional scale, to effectively address the challenges of biodiversity loss and ecological imbalance efforts need to be coordinated globally and unified regulatory measures implemented to avoid e.g. exporting of environmental footprints. The effective assessment of cross-links between regional frameworks and regulations and their global implications could become an additional research focus area in EU Horizon projects. Designing a pragmatic, implementable holistic impact assessment framework remains a challenge for the foreseeable future.

#### **4.02.P-Th445 CSRD and TNFD, learnings from the sustainability reporting journey**

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One of the aims of the European Green Deal is to channel private investments towards activities the EU considers sustainable and therefore a comprehensive regulatory framework on sustainable finance has been established in the past years, which is subsequently entering into force. The Corporate Sustainability Reporting Directive (CSRD) requires European businesses to disclose how they perform according to criteria defined within the European Sustainability Reporting Standards (ESRS). At the same time, the Taxonomy is an additional framework that defines from an European perspective whether an economic activity is considered sustainable or not.

While CSRD, ESRS and Taxonomy are part of a mandatory European framework for companies headquartered within the EU, other voluntary frameworks like the Taskforce on Nature-related Financial Disclosures (TNFD) evolve, with the ambition of a global perspective on sustainability reporting.

An overview of the various frameworks and their interactions will be presented. This will be complemented with concrete examples how businesses currently approach sustainability reporting related to CSRD and TNFD. Key learnings from the current efforts will be provided, e.g. from the locate and evaluate steps of the LEAP (Locate, Evaluate, Assess and Prepare) assessment. Opportunities and limitations in relation to metrics and reporting scope will be discussed.

Taking upstream and downstream reporting into account, metrics require a broad applicability and reliability (e.g. being able to be audited) throughout the value chain to improve outcomes and avoid regretful substitutions. Therefore, a solution-oriented collaboration between regulators, academia and industry on these metrics is suggested, including the opportunity for providing feedback and regularly re-evaluating metrics in terms of legal compliance and financial feasibility.

#### **4.02.P-Th446 Increasing transparency of the environmental profile of agriculture to inform sustainability improvements and environmental protection goals with a pragmatic two-phase methodology**

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Agricultural systems are complex, with many interacting parts, and it is therefore crucial to take a holistic approach to the multiple tools and their wider impacts for a more cohesive understanding of changes in practice. To be able to increase the sustainability of agricultural systems, including their essential ecosystem services and biodiversity, a broad and holistic perspective is required, especially to avoid unintended trade-offs and consequences.

Within this work, a methodology is suggested that builds on the environmental categories of environmental risk assessment processes for plant protection products and uses a rapid evidence approach (REA) to create a broad perspective of the various interactions that can be expected from different agronomic tools.

As a second step, the environmental implications of production systems were investigated. For this purpose, findings from the first step were combined with an additional scientific literature research to fill remaining gaps. This approach supports decision-making by disclosing both benefits and limitations, and may allow further exploration of synergies and trade-offs in agricultural production systems. This can inform cost-benefit frameworks to improve sustainability using key environmental metrics. While ecotoxicological assessments for chemical products play an important role, it is important to consider them in relation to the ecological implications of other decisions taken by a farmer in the field.

Both, the effectiveness of the practices at managing weeds, pests and diseases, and the environmental implications of applying corresponding tools require consideration. The findings can also inform specific protection goals for environmental risk assessments, to ensure that protection goals in terms of biodiversity and ecosystem services will be not undermined by the use of alternatives that are not assessed within a corresponding framework.

In the future, to enable outcome-based business models, which can be more inclusive of biodiversity and ecosystem services outcomes, this transparency can steer decision-making and reduce potential trade-offs between the different tools used in the field.

#### **4.02.P-Th447 Linking Freshwater Mussel Habitat Conditions to Restoration Outcomes and Ecosystem Services**

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Native freshwater mussels are among the most imperiled taxa globally. Once prevalent in the landscape, anthropogenic stressors such as habitat alteration, contaminants, and climate change have led to steep population declines. Such declines have altered habitat functionality through the loss of ecosystem services they provide, such as water quality improvements, sequestration/processing of nutrients, stream stability, and diverse food web structure. This project aims to restore mussel assemblages and their ecosystem services, as well as provide systematic habitat assessments for locations of variable land usage. We studied the Clinton River, an urban watershed river near Detroit, Michigan, USA, with varying water quality issues, strong urban influence, and wastewater input. In the initial phase of the study, we conducted a field study that integrates water quality, habitat conditions, and in situ mussel exposures to assess the potential role of contaminants and habitat stressors on the suitability for mussel restoration. The field study incorporated measures of sediment and water contaminants, water quality analysis, passive samplers, water quality sensors, and monitoring native Fatmucket Mussel placed in situ. The native Fatmucket mussel, *Lampsilis siliquoidea*, was placed in "silos" in the river to assess their survival and growth in the river system. Contaminants were measured in sediment, water, mussel tissue, and passive samplers. Per and polyfluoroalkyl substances (PFAS) were measured using passive samplers. Water samples and mussel tissues were measured for pharmaceuticals. These data will be used to inform the placement of mussels in the river in 2024 and to predict the likelihood of restoration success. Ultimately, we seek to re-establish diverse representative mussel assemblages and evaluate the resulting ecosystem services provided by freshwater mussels including the impacts on water quality and increased macroinvertebrate diversity.

#### **4.02.P-Th448 Using DECOTABs to evaluate organic matter decomposition by aquatic hyphomycetes in pesticide-contaminated environments**

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DEcomposition and CONsumption TABLEts (DECOTABs) contain cellulose powder embedded in an agar matrix that were developed with the aim to standardize the assessment of leaf decomposition in aquatic environments. The decomposition of organic matter helps to understand how efficiently decomposers (such as fungi) contribute to nutrient cycling, which is a central ecosystem function. Fungi, such as aquatic hyphomycetes, are important members of the decomposer community. For this reason, it seems logical, to better understand the responses of these organisms and the functions they perform under exposure to chemicals. Despite this, fungi are not included in standardized testing schemes, even if in environments contaminated, for example, by pesticides, fungal performance may be altered. Considering the high representation of fungicides in the pesticide sales in the European Union and the fact that aquatic fungi are, due to their mode of toxic action, very likely to be affected, the aim of this study is to evaluate fungicide effects on the decomposition rate of these organisms using a modified version of DECOTABs. For this, we apply the fungicide trifloxystrobin at 0, 5, 25, 125 and 625 µg/L. In addition, we adjusted the compositions of DECOTABs by increasing the share of leaf powder (LP) in the agar matrix increasing ecological relevance. Using this design, the DECOTABs will contain 100% cellulose powder (CP), 66% CP/33% LP, 33% CP/66% LP and 100% LP. Three species of aquatic hyphomycetes will be inserted into the test containers: *Alatospora acuminata*, *Articulospora tetracladia* and *Tetracladium marchalianum*. The tests are planned to last 21 days and decomposition will be assessed through the weight loss of the DECOTABs. The abundance of aquatic fungi will be estimated using species-specific quantitative Polymerase Chain Reaction (qPCR) assays. Through this approach, we hope to increase the substrate complexity of DECOTABs approaching natural leaf litter and by this increase ecological relevance. With the homogenisation of large amounts of leaf litter during the production of LP, we still generate a rather standardised substrate using *Alnus glutinosa* powder. The latter might be an important step in paving the way for including aquatic fungi in standardised ecotoxicological testing schemes.

#### **4.03.P Characterization, Testing and Assessment of Complex Substances (MCS, UVCBs & MOCS)**

##### **4.03.P-We280 Tripartite Perspectives on Challenges and Opportunities For The Environmental Testing and Assessment of UVCBs**

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Complex substances such as multi-constituent substances (MCS) and unknown or variable composition, complex reaction products, or biological materials (UVCBs) usually result from the industrial processing or extraction of natural substances or from chemical reactions. Because of the variable and complex nature of source materials, and the potential variability inherent to production processes, these substances can contain many, sometimes uncharacterised, constituents whose concentrations may vary between batches. UVCBs make up approximately 20-25% of chemicals registered globally. To identify and advance the various challenges associated with UVCB testing and assessment, the Health and Environmental Sciences Institute (HESI) organized an international workshop on *Exploring the complexities of UVCB testing and risk assessment* that took place on September 18<sup>th</sup> and 19<sup>th</sup>, 2023, in Reykjavik, Iceland. The 25 workshop participants represented academia, governments, and agencies, as well as, the private sector, and originated from Belgium, Canada, Denmark, France, Germany, the Netherlands, Norway, the United Kingdom, and the United States of America. The HESI UVCB workshop was aimed at initiating multi-sectoral, tripartite discussions on the advantages and disadvantages of the whole substance vs. representative constituent testing and assessment approaches, at identifying further research needs, and at establishing potential consensus for solutions for UVCB environmental risk assessment. Ultimately, the insight from the workshop contributed to keep refining and further strengthen the exposure-centric tiered approach developed for the environmental risk assessment of UVCBs and multi-constituent substances.

##### **4.03.P-We281 Determining Whole UVCB Mineralisation & Constituent Specific Primary Degradation in a Modified OECD 301 Ready Biodegradability Test of an Essential Oil**

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Standard OECD 301 guidelines are presently not suitable for assessing the ready biodegradability of “Substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs)” such as essential oils. The main challenges are that (1) respiration alone is insufficient to prove degradation of the entire UVCB mixture, (2) volatile constituents are easily lost from standard test systems, and (3) hydrophobic constituents can be difficult to dose and can also sorb to the plastic components of certain test systems.

The aim of this study was to assess the ready biodegradability of an essential oil based on a biodegradation test that yields whole UVCB mineralisation (oxygen respiration) as well as constituent specific primary degradation (substrate depletion). The determination of whole UVCB mineralisation requires gastight and inert test flasks, aerobic conditions, and contactless oxygen



measurements. The determination of primary degradation requires alignment of the test system with constituent specific analysis.

The biodegradation test was conducted in glass flasks with PTFE-lined gastight caps. Activated sludge was used as inoculum (30 mg suspended solids/L) at a test concentration of 10 mg/L black pepper essential oil. Test systems were incubated at 20°C for 28 days with daily oxygen measurements. At pre-determined time points, subsamples were taken for solid-phase microextraction (SPME) GC-MS analysis and constituent specific biodegradation was determined based on peak area ratios between triplicate biotic and abiotic test systems.

Whole UVCB mineralisation and primary biodegradation of all major constituents fulfilled the OECD 301 pass levels for ready biodegradability. A respiration of 77% of the Theoretical Oxygen Demand indicated that most of the UVCB mixture was mineralised, and the GC-MS analysis proved primary degradation of all 8 constituents specified in the certificate of analysis and of additional 14 minor constituents.

#### **4.03.P-We282 Applying the principles of grouping and read-across to different lines of evidence to support the development of an ecotoxicity testing strategy for hydrocarbon UVCBs**

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Substance grouping and read-across are commonly employed to reduce the amount of testing required for chemical registrations under the EU REACH regulation (EC 1907/2006). These approaches use relevant information from analogous ('source') substances to predict the properties of 'target' substances. While available guidance tends to be tailored for mono-constituent substances, similar concepts can be applied for ecotoxicity testing of more complex UVCB substances (substances of Unknown, Variable composition, Complex reaction products, or Biological origin). In the present effort, we apply multiple lines of evidence, aligned with read-across principles, to design a testing program for hydrocarbon UVCBs.

Petroleum substances (PS) are comprised of complex combinations of hydrocarbons for which the precise identity of all constituents is usually unknown, and the composition is often variable. Despite these uncertainties, PS may be categorized according to their manufacturing process and their broadly similar physical-chemical properties. Using a comprehensive GCxGC analytical program, the composition of each hydrocarbon UVCB substance can be broken down into relative concentrations of groups defined by carbon number and chemical class, termed hydrocarbon blocks (HCB). Using non-polar narcosis as the common primary mechanism of toxic action for hydrocarbons, toxicity would be correlated with bioavailability. It is then possible to use representative constituents for each of the relevant hydrocarbon blocks to estimate the toxicity of the whole substance.

For the testing strategy, we determine the "worst-case", or most toxic, sample using two independent methods: (i) an experimental biomimetic extraction-solid phase microextraction (BE-SPME) approach which mimics aqueous exposure to PS in the environment; and (ii) the PetroTox QSAR model that utilizes two sub models (i.e., target lipid model and an oil dissolution model) to estimate PS toxicity to ecological receptors. The outcomes of these two exercises allow for the selection of a "worst-case" PS sample for testing and account for the range in compositional variability across samples. The concepts and various lines of evidence applied here will support testing strategies for multiple PS categories and could inform chemical prioritization initiatives or other regulatory assessments of UVCBs.

#### **4.03.P-We283 Contrasting, Characterizing and Predicting the Toxicity and Risk of Natural Complex Substances**

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Testing of Unknown or Variable Composition, Complex Reaction Products or Biological Materials (UVCB) poses a regulatory challenge. Indeed, Natural Complex Substances (NCS1) are characterised at >90% while NCS2 are UVCB of which often <50% of the composition is known. Based on two projects combining complex mixture toxicity testing and toxicity predictive approaches, this work aims to show the potential of *in silico* methods for NCS1&2 toxicity prediction and environmental risk assessment.

Project 1 consisted to test daphnids (pelagic) and *Lumbriculus* (sediment) sensitivity to an equimolar mixture of 4 constituents with log P values of 1, 2, 3 and 4. The WAF (Water Accomodated Fraction) *in silico* method considering constituents chemical activity accurately predicted the acute and chronic ecotoxicities in the pelagic phase as well as the chronic toxicity in the sediment phase, when modified to include the Equilibrium Partition (EP). Also, EP method accurately predicted constituents concentration in sediments. Finally, Predicted Environmental Concentration (PEC) were estimated using the chemical activity method and the PEC/Predicted No Effect Concentration ratio characterised as activities.

In project 2, the NCS2 Galbanum resinoid was fractionated resulting in a volatile, a non-volatile, and an inert fraction. Volatile and non-volatile (including the inert one) fractions represented up to 35% and 65% of the whole substance, respectively, with specific composition. Acute and chronic sensitivity of algae and daphnids to the whole substance and each fraction was

determined experimentally and predicted using additivity and *in silico* WAF approaches. The WAF provided the best results for the whole substance when based on the theoretical composition for acute toxicity and based on the refined composition for chronic toxicity, when analytical monitoring exhibited a compound loss during the test.

In summary, the *in silico* WAF approach was promising to predict acute and chronic ecotoxicity of NCS1&2. NCS1 example showed that mixture environmental risk assessment in multiple compartments is possible using a chemical activity approach. Modelling allow to identify the constituents leading NCS toxicity but experiments allow to identify constituents behaviour over-time and to refine composition in calculation. For now, modelling and experimental approaches are complementary. The approach reproducibility to other NCS2 is undergoing on clary sage concrete.

#### **4.03.P-We284 Predicting Dermal and Inhalation Exposure from Mixtures: Two Case Studies**

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Many commonly used consumer and industrial products are chemical mixtures. Evaluation of human exposure requires knowledge of a substance's physico-chemical (PC) properties and components of the mixture can change the effective properties of the chemical of interest in the mixture. It is desirable to be able to confidently predict the properties of constituents of interest in a mixture to save time and money and to avoid the environmental impacts of carrying out experimental procedures. We have developed a new tiered system for estimating the properties of known constituents in defined mixtures. The framework is freely accessible in the Exposure And Safety Estimation (EAS-E Suite; [www.eas-e-suite](http://www.eas-e-suite)) on-line platform. Here we apply this framework to two case studies for predicting human exposure of substances originating from a mixture source: inhalation exposure of diesel fuel spilled in an indoor environment and dermal exposure of a transdermal drug from a skin cream product. PC properties including vapour pressures, partition ratios, and dermal permeation coefficients ( $K_p$ ) are predicted using different models and compared to exposure concentrations predicted using experimental PC properties. The different models for predicting PC properties include COSMOtherm, Raoult's law, ten Berge QSPR model, Atoke QSPR model, and several newly developed IFSQSAR/ppLFER models including the log-linear ppLFER, log-linear with Binary Interaction Parameters ppLFER, log  $K_p$  Thermodynamic Cycle ppLFER, and the log  $K_p$  Thermodynamic Cycle treating penetration enhancers pp-LFER. PC property predictions from the different methods are then used to parameterize the Risk Assessment IDentification And Ranking – Indoor Consumer Exposure (RAIDAR-ICE) model to examine the accuracy of predicted exposure concentrations resulting from different PC prediction methods. Results show that while it did not make much of a difference whether mixture effects were accounted for in the diesel fuel, the skin cream mixture showed a large variability in exposure concentrations, up to a factor difference of 130 between the exposure concentration predicted using  $K_p$  from a PC prediction method and predicted using a measured  $K_p$  value. Finally, a sensitivity analysis demonstrates how partitioning behaviour changes due to mixture effects for each of these mixtures which are very different in nature.

#### **4.03.P-We285 Environmental Classification of Ferromolybdenum - Impact of Copper Impurity Levels**

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Ferromolybdenum, also named FeMo, is an alloy manufactured and used in both the powder and massive form. Alloys are considered as special mixtures, and for the purpose of a hazard assessment and classification under the EU Classification, Labelling & Packaging of Substances and Mixtures (Regulation No. 1272/2008), the mixture rules as outlined in this Regulation are applicable for FeMo.

Copper - an element for which an EU harmonized classification of Aq.Acute 1 (M=10), and Aq.Chronic 1 (M=1) has been established for the powder form – is the main environmentally hazardous impurity relevant for classification assessment: its maximum concentration exceeds the threshold above which it is required to include the concentration of a hazardous impurity in hazard classification calculations. The harmonized Cu-classification, however, is only applicable on Cu in FeMo when there is evidence that Cu-release rate from FeMo-powder is similar or lower than that of Cu powder. In the absence of such information the worst-case assumption that all Cu is bioavailable must be followed, resulting in an Aq. Chronic 2/3 classification for FeMo with a Cu content of >0.4% and >0.04%, respectively.

The release of Cu from FeMo was therefore assessed with three different FeMo-powders from various producers, with each sample having its specific impurity levels, particle size distribution and specific surface area. 24h-screening Transformation/Dissolution protocol tests at pH 6 and 8 were performed and Cu-release was determined, with the highest release observed at pH 6.

Average Cu-content of the FeMo samples was 0.31% (CV: 26.1%), with an average Cu-release of 0.372 mg Cu/m<sup>2</sup> at pH 6. Normalization of this value to a Cu-content of 100% and taking an extended exposure period of 7 days into account (assumption: linear time-dissolution relationship) resulted in an average 7day release of 0.78 µg Cu/mm<sup>2</sup>. This value falls within the range of known 7-day Cu-release rates for copper in massive, powder wire or granulated forms (0.41 – 1.83 µg Cu/mm<sup>2</sup> at pH 6). Equivalence in Cu-release from FeMo and Cu-powder is thus established, and consequently the harmonized classification of Cu-powder is valid for the Cu-fraction in FeMo. Application of the CLP-mixture rules on FeMo results in a

no-classification of massive FeMo, and FeMo-powder containing < 0.25% Cu. FeMo-powder containing 0.25% - 2.5% Cu will be classified as Aq. Chronic Cat.3 in the absence of further data.

#### **4.03.P-We286 Navigating the Complexity: Challenges in Ecotoxicity Testing of UVCB Substances**

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The European REACH Grease Thickeners Consortium (ERGTC) has registered several grease thickeners under the REACH regulation. Most of these substances are poorly soluble, surface active, and/or Unknown or Variable compositions, Complex reaction products and Biological materials (UVCBs), falling under the category of difficult-to-test substances. Testing these substances, especially for aquatic toxicity, poses considerable challenges, including the preparation of test solutions to achieve and maintain the required test concentration and analysis of the test item during exposure.

As part of extensive testing programs to improve the data quality in the REACH registration dossiers, the ERGTC has developed an approach focused on preparation of Water Accommodated Fractions (WAFs) to meet some of these challenges with UVCBs. Preliminary trials were conducted to determine the maximum dissolved concentration achievable in the test solution and the procedures required to obtain this concentration were established.

For test solution preparation, adjustments were made to the test media preparation methods. One of the critical factors was stirring duration which varied between 24 hours and 7 days, depending on the substance's properties. Solutions requiring over 72 hours of stirring were initially prepared in reversed-osmosis water, followed by filtration and the addition of nutrients to form the test media, due to concerns about the test medium stability over the combined stirring and exposure duration.

A further challenge arose from the substances' poor solubility and analytical limitations, affecting the measurement of test item concentration in test media. Although solvents should be avoided during test media preparation as this may lead to testing concentrations above the solubility limit, a solvent evaporation approach was considered suitable to overcome the issues with preparation of low volumes of media at low concentrations and to enable analytical verification of the test item levels.

This poster will provide an overview of the challenges during ecotoxicity testing and outline the 'best practice approach' developed from the experiences gained with the grease thickeners. These approaches can be used to generate reliable data for risk assessment and the formulation of effective environmental management strategies.

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#### **4.03.P-We287 Optimising Testing Strategies for UVCB Substance Categories to Minimise Vertebrate Testing for Aquatic Toxicity**

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Under the EU REACH Regulation high volume chemicals must provide a dataset of aquatic toxicity for acute and chronic toxicity data covering 3 trophic levels. One way of optimising efforts and reduce unnecessary animal testing is by read-across of relevant data from similar substances. This approach is particularly strong when using several analogue substances (category approach) instead of only one (analogue approach). However, this approach requires the demonstration of structural similarity between the substances in the category, as well as evidence of similar properties, making it particularly challenging for UVCBs due to the uncertainties and variability of their composition.

We present a successful strategy for category read-across within categories of olefinic streams, using several lines of evidence integrated in a tiered approach, in order to assess the aquatic toxicity of those substances with considerable reduction in the number of tests needed. The strategy uses: a) detailed compositional analysis, b) toxicity screening via alternative approaches and c) testing of the UVCBs in the category, starting with algae and proceeding to invertebrates and fish. The strategy has been designed so that all the substances in a category are screened with the lower tier approaches, thereby "bracketing" the ecotoxicity covered by all the chemical space of the category. If lower tier data are consistent, this builds confidence that allows to read-across for higher tier data with minimal testing. The advantage of this multi-faceted approach is that the difficulties in the read-across, caused by the complex composition of the UVCBs assessed, are balanced by the coherence in several different types of activity data, indicating that the aquatic toxicity can be well predicted across category members. In addition, because the lower tier data are all non-vertebrate tests, and the necessary higher tier tests are minimal, the reduction in the number of vertebrate tests conducted is substantial. The approach has been piloted with several categories of hydrocarbon streams, and could potentially be expanded to other UVCBs.

#### **4.03.P-We288 Application of PBT and PMT Criteria to Complex Substances: A Case Study of Essential Oils**

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Essential oils (EOs) are part of the Natural Complex Substances (NCS) family often referred to as UVCB substances (substances of Unknown or Variable composition, Complex reaction products or Biological materials). Assessing their risks and properties is not straightforward, since complex substances typically contain a large and variable number of constituents, and often a high concentration of unknown constituents.

The update of the CLP regulation in 2023 introduced PMT and PBT hazard class and criteria, plus the notion of MOCS (more than one constituent substance). This regulatory change has led to a new approach to the evaluation of persistent (P) and bioaccumulative (B) criteria for UVCBs.

PBT and PMT classification in CLP by 2025 will have to be dealt with the PBT assessment already required for substances registered under Annex VIII of REACH (above 10 t/y). The CLP regulation does not require testing, but use of existing data on biodegradation, log  $K_{OW}$ , and  $EC_{50}$  on aquatic invertebrate and on algae are required in Annex VII and will therefore be available as screening data to be used in the PBT assessment. Thus, to satisfy CLP regulation, a PBT assessment will be required for essential oils already registered under REACH. However, the absence of screening information on the mobility criteria (log  $K_{oc}$ ), only required in annex VIII of REACH regulation, will make PMT assessment difficult for the majority of EOs.

First, our study details and compares the current approach used and described in ECHA and industry guides (IFRA / EFEO) with the MOCS approach to PBT and PMT assessment. We discuss the impact of assessing the P, B, and M criteria on the known constituents, but the T criterion on the whole substance.

In the second step, examples of substances with available data demonstrate that the constituent approach and experimental data obtained on the whole substance are sometimes not concordant for the P and B criterion. We conclude that interactions between constituents in natural substances are more complex than a simple additivity effect.

A proposed amendment to exempt "substances of renewable botanical origin that are not chemically or genetically modified" from the use of the MOCS approach is currently under discussion at European level. We hope that these data will inform stakeholders and support discussions on the application of the MOCS approach to natural substances.

#### **4.03.P-We289 Investigation Into the Impact of a Substances' Physicochemical Properties on its Suitability to be Dosed at High Flow Rates into Aqueous Media Using Saturator Columns.**

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A saturator column is a device used to generate a substance solution at up to its maximum solubility (in, for example, water), without the inclusion of a separate solvent to aid dissolution. The use of such devices to generate test solutions is one of the recommended methods in OECD guidance document N.23 for the aqueous-phase aquatic toxicity testing of difficult test chemicals. The use of saturator columns is not generally recommended when testing complex mixtures of chemicals (e.g. UVCB's) due to the potential disparity in depletion rates for the different component chemicals, however, generating a constant flow of aqueous media, dosed at the functional limit of solubility (for all individual components of a mixture) without the use of a solvent is not otherwise easily achieved.

To conduct toxicity testing at concentrations approximating the solubility limit of a substance using a flow through study design (for example in a fish early life stage toxicity test, or an aquatic exposure bioaccumulation study), the saturator column(s) flow rate needs to match that of the flow through test system.

This study investigates the saturator column eluate concentration of a range of chemicals with varying physicochemical properties, at column flow rates appropriate to the aforementioned study types. The study aims to better understand the physicochemical characteristics of a chemical that make it a viable target for use in dosing into high flow rate aquatic toxicity tests using saturation columns. The components are tested individually and as a mixture to determine if understanding of the individual component properties is applicable to complex mixtures.

#### **4.03.P-We290 Studying the Effects of Oil Sands Process-Affected Water Composition on PAH Henry's Law Constants**

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The Canadian oil sands industry generates large volumes of oil sands process-affected water (OSPW) as a waste byproduct. These waters contain significant amounts of naphthenic acids, along with a variety of inorganic salts and other

solutes. Environmental monitoring of such waters requires knowledge of the properties of the various OSPW constituents. In particular, the Henry's law constant is a key property for the monitoring and assessment of atmospheric emissions originating from OSPW.

A key question is whether the Henry's law constants of OSPW solutes can be approximated by their corresponding values in pure water, an assumption utilized by most prediction methods. This study addresses this concern by studying the extent to which the Henry's law constants of PAHs in OSPW are affected by the constituents contained within OSPW, namely naphthenic acids and inorganic ions. We present here results from COSMO-RS modelling of Henry's law constants for a set of 16 polycyclic aromatic hydrocarbons (PAHs) in OSPW, in the presence and absence of typical OSPW constituents. The absolute deviation in predicted Henry's law constants between pure water and the simulated OSPW ranged from 0.011 to 0.017 log units. Our results demonstrate that the use of Henry's law constants predicted assuming a pure water medium is an acceptable approximation for modelling the atmospheric emission of OSPW constituents. Temperature variation effects are shown to be significantly more important in terms of influencing the Henry's law constant compared to the influence of any OSPW constituents at the concentrations that they are found in typical OSPW.

#### **4.03.P-We291 Towards Improving the Reproducibility and Linearity of the Preparation of Water Accommodated Fractions of Esterification Reaction Products**

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Unknown or variable, complex reaction products and biological materials (UVCBs) are a developing area of concern, comprising up to 40% of new chemicals registered in Europe. Of particular interest are UVCBs prepared from esterification reactions with starting materials such as pentaerythritol and ethylene glycols; their unique structures allow the production of a myriad of materials for use in a wide range of industries (adhesives, lubricants, polishes, fertilizers, and coating products, to name a few) dependant on the type of free acid used in the reaction. Poorly soluble and hydrolytically unstable, the physicochemical properties of these UVCBs pose additional challenges when assessing environmental risk in comparison to other multi-component substances.

The largest challenge in assessing the aquatic toxicity of these types of UVCBs is their dosing, for which the Water Accommodated Fraction (WAF) has been the standard regulatory practice for over thirty years. Although WAFs are the principal method of dosing difficult-to-test substances, the reproducibility between preparations is generally considered poor despite attempts at standardisation. The competing thermodynamics of phase equilibration and hydrolytic instability have historically yielded low reproducibility between saturated solutions. The complexity of the system is often further exacerbated by the creation of stable suspensions that can overestimate the saturation concentration.

In this work, we present a novel approach to WAF preparation which aims to improve reproducibility by shortening equilibration times and reducing the formation of colloidal matter via a solvent-dosed solid support membrane. This hybrid system utilises the ease of solvent dosing with the fast equilibration and reduction of suspension formation observed in passive dosing, all whilst maintaining the principles of WAF preparation. We present the degradation and phase equilibration kinetics for a range of esterification reaction products, with recommendations for obtaining a linear response to WAF loading using solvent-dosed solid supports.

#### **4.03.P-We292 Searching for slowly-biodegrading constituents using whole petroleum substance biodegradation testing with deconvolution and non-target analysis of GCxGC-FID/TOFMS data**

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Petroleum substances contain thousands of constituents that biodegrade at widely varying rates. Recently, we developed a workflow to search for slowly biodegrading constituents using whole petroleum substance biodegradation testing with constituent-tracking methods, based on analysis by comprehensive two-dimensional gas chromatography coupled to flame ionization detector (GCxGC-FID) and to high-resolution time-of-flight mass spectrometry (GCxGC-HR-TOFMS). However, chromatographic coelutions challenged our efforts to quantify, track, and identify constituents in these highly complex UVCBs. In the present study, we re-design the workflow to include deconvolution and non-target analysis approaches to improve the detection, quantification, and identification of slowly-biodegrading constituents. The screening method detects, quantifies, and identifies constituents that exceed the following criteria: (i) the constituent concentration in the original product exceeds 300 mg g<sup>-1</sup>; and (ii) the percentage of remaining (non-degraded) mass exceeds 30% after 64 days of biodegradation. We test the screening method on a diesel fuel previously analyzed by GCxGC-FID/TOFMS. We found 50 constituents that met the screening criteria. Among these, 43 constituents (86%) are interpreted as C<sub>15</sub>-C<sub>23</sub> two-ring naphthenes, of which 30 likely contain a quaternary carbon. Five constituents (10%) are C<sub>21</sub>-C<sub>23</sub> acyclic isoprenoids, and two constituents (4%) were not successfully interpreted. Example results are shown for a peak interpreted as a C<sub>15</sub>-naphthene. The re-designed workflow improves on the detection, quantification, and identification of peaks, compared to the previous method. This conclusion is illustrated by the finding that most of the screened constituents are identified as naphthenes containing quaternary carbons, a structural feature associated with recalcitrance to biodegradation. Information gained from the screening method can be used to

prioritize further testing of constituents expected to biodegrade slowly. The data analysis workflow can be extended to other endpoints requiring time-lapsed information, such as bioaccumulation potential.

#### **4.03.P-We293 Application of MOSH/MOAH GCxGC methods to support bioaccumulation testing of hydrocarbon UVCBs in fish**

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The assessment of chemical bioaccumulation in fish is an important component of global chemical assessment and regulatory programs. Bioaccumulation assessments for petroleum substances remain difficult because (i) they are comprised of complex and variable combinations of hydrocarbons (i.e., UVCBs); and (ii) there are limited test data for the thousands of possible hydrocarbon constituents in petroleum substances, due to limited commercial availability of the individual constituents and their incompatibility with standard test systems. To deal with these issues, we are running whole substance OECD 305 dietary bioaccumulation test paired with supporting two-dimensional gas chromatography (GCxGC) analysis to analytically track individual constituents, or groups of constituents, over time. This approach of generating constituent-level information in a whole substance test design has been successfully applied in seawater biodegradation tests. However, the increasing complexity of biological sample matrices (e.g., due to lipids and other biogenic compounds) can present challenges for adequate peak separation and identification. In the present study we aim to optimize and test analytical methods needed to characterize the dietary accumulation of middle distillate oils in trout and highlight analytical challenges associated with the generation of bioaccumulation data using GCxGC analysis. To improve peak separation and recovery, fish samples were subject to alkaline digestion and epoxidation steps to remove residual lipids and polyenes which thereby reduced analyte interference and carryover between chromatogram runs. Addition of an HPLC pre-fractionation step to separate saturated (MOSH) and aromatic (MOAH) hydrocarbon fractions also greatly reduced peak overlap and interference. Preliminary analyses revealed the presence of MOSH contaminants in control fish, possibly from the prepared fish food or sample homogenization procedures. Food-derived oils may naturally contain MOSH/MOAH and are commonly added to fish feeds in bioaccumulation studies. Caution should therefore be taken in sample preparation to avoid MOSH/MOAH cross-contamination. Following a blank-correction, apparent detection of C10-C15 hydrocarbons in exposed trout was observed. These data are being used to optimize the sample processing and analytical methods so that additional analyses can be performed to determine the bioaccumulation and biotransformation behaviour of these compounds.

#### **4.03.P-We294 Outcomes from the 2023 Concawe workshop “Analytical technology exchange to meet health & environmental regulatory challenges for UVCBs”**

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The EU REACH Regulation requires registrants of substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs) to provide detailed and comprehensive analytical information on the constituents present in these substances. Concawe currently manages over 140 registered hydrocarbon UVCB substances grouped into multiple categories ranging from light (e.g., naphtha) to heavy (e.g., bitumen) substances. Comprehensive substance characterization is required both to assess the potential environmental and human health impacts of these substances and to assess the degree of similarity between substances. The latter is particularly important so that the results obtained from toxicity tests can be applied from one substance to another (i.e., read-across). In addition, targeted analysis of specific constituents of concern is also important. Recognizing the challenges to characterize UVCB substances, Concawe convened a workshop for analytical chemists, (eco)toxicologists, and product stewards from industry, academia, and regulatory authorities to identify the state of the science and paths forward in defining the composition of petroleum substances for the purposes of hazard assessment.

The Concawe workshop enabled cross-sector and cross-disciplinary exchange on the need for and methods of identification and quantitation of UVCB substance constituents/ constituent groups. As will be shared in the poster, the discussions started with clarifying the regulatory requirements for health and environment and the need for additional analytical data. Several laboratories then presented their current and evolving analytical technologies to support comprehensive description of constituents / constituent groups and to improve targeted resolution and constituent identification. Finally, the analytical methods and their potential were assessed against regulatory requirements to identify the opportunities and gaps. The outcomes of this workshop will be used as guidance for experimental interrogations of a set of UVCB substances by Concawe to understand the capabilities and limitations of current technologies to deliver the type of analytical information being requested for human health and environmental hazard assessment.

#### **4.03.P-We295 Development of a Pilot Database of UVCB Chemical Characterization Information**

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Substances of “unknown or variable composition, complex reaction products or biological materials” (UVCBs) pose

challenges to risk assessors, registrants, scientists, and regulators. To address these challenges, the tripartite UVCB Committee of the Health and Environmental Sciences Institute (HESI), is developing an exposure-based, tiered approach for UVCB prioritization and risk assessment. The first step of the proposed approach (aka Tier 0) involves “Tier 0 information” that can be used to prioritize UVCBs and streamline their safety evaluation. Tier 0 information includes exposure, composition, and hazard data such as use profiles, production volumes, physicochemical properties, and (eco)toxicological data. Despite the prevalence of UVCB substances in the chemical space, there is no public-facing database specifically for relevant chemical characterization data for UVCB substances. Thus, there is an opportunity to develop a fit-for-purpose, publicly accessible database addressing this need.

To assess feasibility and develop robust/efficient methodologies for data collation, a subgroup of the HESI UVCB committee is developing a pilot database of Tier 0 information for ~100 commercially relevant UVCBs. Information has been collected from scientific publications, databases, and reports, including substance identifiers (i.e., CAS Registry numbers, EC Numbers, names), structural descriptors, registration data, physicochemical properties, use/exposure profiles, and hazard data for whole substances and representative constituents. Methods and challenges encountered have been documented for reproducibility and improvement in future data collection efforts. Steps to automate the data mining processes were investigated to aid in database maintenance and future expansion efforts.

This exercise both demonstrates the feasibility of, and highlights the challenges associated with, developing and potentially scaling up such a database. Moreover, this effort progresses the implementation of a UVCB risk assessment framework, providing information that can be utilized by registrants, risk assessors, and regulators for prioritization, risk assessment, and read across. Obtaining buy-in from industrial and regulatory partners will be key to further the data collection process and realization of the full potential of such a database.

*Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the US EPA.*

#### **4.03.P-We296 A Linear Regression Model for Predicting COSMO-RS Sigma Profiles**

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Linear regression models can be very useful for predicting chemical properties with high environmental significance. Quantitative structure-activity relationships (QSARs) and linear free energy relationships (LFERs) are examples of linear regression type models often used in environmental science to predict properties such as partition ratios or biotransformation half-lives.

Sigma profiles are chemical properties that have a graphical form which show the distribution of local charges over the molecule's surface area. Andreas Klamt has shown that sigma profiles can be used to characterize any chemical or mixture of chemicals and has used this as the basis for developing the statistical mechanics-based COSMO-RS theory. COSMO-RS has been implemented into the software COSMOtherm for predicting chemical properties such as partition ratios, solubilities, vapour pressures, Henry's law constants (HLCs), and others.

In this study, a multi-parameter linear regression (MRE) model has been developed using HLCs as descriptors to predict sigma profiles of chemicals. Predictions can be made for pure chemicals or for mixtures including Multi-Constituent Substances (MCS), More-than-One-Constituent Substances (MOCS), and substances of Unknown or Variable composition, Complex reaction products, and Biological materials (UVCBs). In this work, sigma profiles were predicted for a 49 neutral organic compound test set using the generated MRE equations. Sigma profiles were then indirectly input into COSMOtherm and used to predict partition ratios for a group of 264 neutral organic compounds in a medium containing a binary mixture of water and each of the 49 test set compounds. R<sup>2</sup> values for partition ratios predicted using the MRE-generated sigma profiles compared to partition ratios from actual sigma profiles ranged from 0.8794 for the 264 compounds in a 1-nonanol/water medium to 0.9969 for the same set of 264 compounds in an acetonitrile/water medium. The average log RMSE for the partition ratios was 0.48.

These results are promising with respect to the use of experimentally determined HLCs (which can be converted from vapour pressure) to characterize relevant properties of environmental media, especially for UVCB substances, without knowing their actual composition.

#### **4.03.PC Characterization, Testing and Assessment of Complex Substances (MCS, UVCBs & MOCS)**

#### **4.04 Effect Modelling in Regulatory Science: In the Service of Environmental Risk Assessment and Risk Management?**

##### **4.04.T-01 Three Perspectives on Model Calibration for Ecological Risk Assessment**

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Mechanistic effect models are useful tools to support environmental risk assessment of chemicals. The development and application of mechanistic models require decisions on the model type, model structure and parameterization. "To calibrate or not to calibrate" is still under debate, especially in the field of mechanistic modelling. In this paper, we discuss three complementary perspectives on model calibration, including model selection and parameterization. They differ in their way to deal with prior knowledge and with data for calibration and validation. We discuss implications on model performance and transferability to different application scenarios. Furthermore, we discuss implications on the quantification of model output uncertainty.

With this presentation, we hope to bridge between different modelling communities from mechanistic to statistical modelling and machine learning fields. We provide motivation and guidance for the use of transparent concepts and reproducible procedures to include different sources of information for decision support in environmental risk assessment. We also provide a perspective on future developments to increase the robustness of model predictions and transparency regarding model uncertainties.

#### **4.04.T-02 Parameter Estimates Using the GUTS Modelling Framework: Biological or Statistical Reality?**

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It is common practice in ecotoxicology to derive an LC<sub>50</sub> (the concentration at which 50% of the exposed organisms die, at some specified point in time) from laboratory experiments. This LC<sub>50</sub> is subsequently treated as the single value summary of the test and is considered to be a measure for the toxicity of a compound and is subsequently used to derive a safe environmental concentration. This approach has a number of disadvantages, most notably the time-dependence of the LC<sub>50</sub> and the fact that the LC<sub>50</sub> is only valid for the exposure conditions under which it was derived, usually a constant exposure.

These disadvantages can be overcome with the use of a toxicokinetic-toxicodynamic (TKTD) model for the interpretation of effects. The most elaborate modelling framework that is currently in place is the General Unified Threshold model of Survival (GUTS) modelling framework and currently has a formal status for use in risk assessment by EFSA. Most frequently the GUTS model is used in its reduced form where the external concentration is the driving force for effects. We've applied the standard model to some datasets generated in the laboratories of Wageningen University and Research, that comply with the EFSA standards on data quality to evaluate the outcome. This showed that the best fitting parameter values in the standard settings may significantly underestimate the intrinsic sensitivity of the species in a test.

The main lessons that can be learned from this exercise are:

- Fit the control mortality on the whole dataset
- Check if the obtained parameter settings are plausible
- Non-dedicated experiments will often result in correlated parameter values, hampering the extrapolation potential of a TKTD approach
- If more than one interpretation of the data is possible; explore every interpretation individually

#### **4.04.T-03 Spatio-temporal coupling of soil exposure modelling with toxicokinetic-toxicodynamic models: a case study based on a field trial**

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The environmental risk assessment of plant protection products aims at the prevention of unacceptable adverse effects on the environment. Therefore, the risk assessment for soil organisms, such as earthworms, is based on two key elements: the exposure assessment and the effect assessment which are conducted currently independent from each other. Regarding the exposure, the behavior of an active ingredient in the soil is evaluated and, as a major output, predicted environmental concentrations are estimated. The effect assessment is based on toxicity endpoints derived from the investigation of the concentration–response relationships in laboratory studies or a NOEC in field experiments.

Laboratory tests conducted for earthworm risk assessment are worldwide agreed and standardized (OECD 207 & OECD 222). The standard test species is *Eisenia fetida*, conducted at constant and optimal temperature, with artificial soil and controlled humic conditions. This allows to detect lethal and sublethal effects.

In the field we have different earthworm species like *Aporrectodea caliginosa* and *Lumbricus terrestris* which experience fluctuating environmental conditions, e.g. temperature, humidity and food. In addition this fluctuation changes in depth of the soil column and depend on soil type. Soil type and weather also influence the fate of pesticides in the soil column and have effects on the movement and behaviour of the earthworms and their life-history.



In this approach we added species-dependent movement from Gergs et al. (2022) due to moisture and food gradients as well as life-history development after Dynamic energy budget (DEB) theory to the EEEworm from Johnston et al. (2014, 2015 & 2018). The “FORESEE” model - a spatial and temporal explicit (DEB-)TKTD (toxicokinetic-toxicodynamic) model for earthworms - was provided with fate exposure profiles of carbendazim, FOCUS weather data (see Figure 1) and DEB & DEB-TKTD-parameters for both earthworms derived from the Add-my-Pet database & laboratory tests. The model performance was compared to data from a field study with carbendazim for both species.

The here presented FORESEE model can help to understand effects of environmental fluctuations on earthworm populations. In addition it gives insights in the mechanistic processes during exposure situations and can support field studies by screening exposure scenarios and timing of application beforehand.

#### **4.04.T-04 Linking Landscape Models, Field Data and Pesticide Effects Using Normal Operating Ranges (NOR)**

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Arthropods play diverse roles in agricultural ecosystems, from pests to providers of crucial ecosystem services like pollination and pest control. Assessing declines in beneficial arthropod populations, influenced by factors such as pesticides, is challenging due to variability in inter and intra-annual species abundances. Natural variability in an ecosystem is therefore first assessed using Normal Operating Ranges (NOR) quantifying 'normal' species abundance throughout the year. This concept is then used to link landscape-scale modelling results with field data and pesticide effects. Modelling is performed in the Animal Landscape and Man Simulation System (ALMaSS) from Topping et al. (2003) for the predatory money spider *Erigone atra*. ALMaSS uses landscapes derived from real aerial imagery together with factors like weather, crop management and species behaviour in the simulation. The model-derived NOR results are compared with a pitfall trap field data for *E. atra*. For this purpose, field data surveys are extracted from literature, interpolated, pooled and then used to calculate a data-derived NOR. Data and model-derived NORs show similar patterns during the year and thus indicate comparability between model results and field data. Pesticides are then introduced in the model with different generic properties, like lethality, longevity and application rate to assess effects on species abundance. Effects are analysed according to the EFSA scientific opinion on recovery (2016) based on whether abundance reductions exceed NOR boundaries. Using the Normal Operating Range (NOR) concept can therefore aid the linkage between landscape-scale models, field survey data and assessment of pesticide effects. Abundance results can further be related to their ecosystem function to analyse risk on the level of ecosystem service provision. Future assessment of pesticide effects on a landscape scale can thus be supported by modelling.

#### **4.04.T-05 xLandscape – A Modular Modelling Framework for Landscape-Level Exposure and Effect Modelling and Risk Characterisation**

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More realistic environmental risk assessment of pesticides requires to operate exposure and effect models based on real-world (geo)information. Established models for the different domains need to be technically linked and provided with environmental and agricultural data. To overcome ad-hoc solutions, to assure consistency and to manage complexity, a modular framework has been developed to integrate individual models into a new, larger model of combined capabilities (xLandscape). xLandscape allows to deliver risk assessment endpoints in explicit dimensions and scales as defined in Specific Protection Goals. Here, we introduce the concepts and design of xLandscape and show example model applications.

We exemplarily show how xLandscape models are built and demonstrate simulation results for aquatic and off-field-soil exposure and effect modelling as well as for bee risk scenario development. The results show how new insights into real-world systems are gained and how these complex multidimensional data can be summarised into intelligible endpoints for risk assessment and risk management purposes.

#### **4.04.P Effect Modelling in Regulatory Science: In the Service of Environmental Risk Assessment and Risk Management?**

##### **4.04.P-We297 The MAD book - Acceptability of Regulatory Models**

**Jeremias Martin Becker<sup>1</sup>, Magnus Wang<sup>2</sup>, Alpar Barsi<sup>3</sup>, Udo Hommen<sup>4</sup>, Thomas G Preuss<sup>5</sup>, Oliver Jakoby<sup>6</sup>, Benoit Goussen<sup>7</sup>, Stefan Reichenberger<sup>8</sup>, Melissa Reed<sup>9</sup> and Sabine Duquesne<sup>10</sup>, (1)German Federal Environmental Agency (UBA), Germany, (2)WSC Scientific GmbH, Germany, (3)Board for the Authorisation of Plant Protection Products and Biocides (Ctgb), Netherlands, (4)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (5)Bayer AG - Crop Science Division, Germany, (6)RIFCON GmbH, Germany, (7)ibacon GmbH, Germany, (8)knoell France SAS, France, (9)HSE, United Kingdom, (10)German Environment Agency (UBA), Germany**

In the European Union, mechanistic effect models (MEMs) have to be thoroughly evaluated before they may be used for the regulatory environmental risk assessment of plant protection. MEM evaluation has been mainly described in the EFSA Scientific Opinion on Good Modeling Practice (2014). The evaluation process in that document follows typical steps in modeling, but does not separate the development and the application of a MEM. So far, no fit-for-purpose models other than GUTS and the *Lemna* model have been acknowledged by EFSA. In consequence, often a rapporteur member state has to fully

evaluate a model each time a new application of a MEM has been proposed. This is inefficient and can lead to inconsistency in the evaluation and acceptance, because MEMs for risk assessment are typically not developed for a single application.

Therefore, we suggest to separate the evaluation of a MEM in general and of its application in a specific risk assessment. Additionally, the evaluation of a general MEM may be broken down to building blocks (modules) for specific processes in a model. Accepted modules may then be assembled to a new modelling framework for risk assessment, where only the interplay of the components, its suitability to address a specific question, the environmental scenario and the pesticide-specific information need to be evaluated. We consider such a modelling framework to consist of a fate model with an associated exposure scenario and a coupled MEM with an associated ecological scenario. The MEM is considered to consist of two main parts: The toxicology module simulates the immediate effects of a pesticide on the modelled entity (e.g., decreased nutrient uptake in a TKTD model or decreased survival in a population model), while the ecology module simulates the ecological consequences (e.g., changes in organism growth or in population dynamics).

The proposed evaluation of such a modelling framework, the embedded MEM and its modules allow for more flexibility as compared to the evaluation of a ready-to-use but rigid regulatory model as described in the EFSA scientific opinion on Good Modelling Practice.

#### **4.04.P-We298 The MAD-Book - Potential Applications of Mechanistic Effect Models in Environmental Risk Assessment of Pesticides**

*Jeremias Martin Becker<sup>1</sup>, Magnus Wang<sup>2</sup>, Alpar Barsi<sup>3</sup>, Thomas G Preuss<sup>4</sup>, Udo Hommen<sup>5</sup>, Oliver Jakoby<sup>6</sup>, Benoit Goussen<sup>7</sup>, Stefan Reichenberger<sup>8</sup>, Melissa Reed<sup>9</sup>, Johan Axelman<sup>10</sup> and Sabine Duquesne<sup>11</sup>, (1)German Federal Environmental Agency (UBA), Germany, (2)WSC Scientific GmbH, Germany, (3)Board for the Authorisation of Plant Protection Products and Biocides (Ctgb), Netherlands, (4)Bayer AG - Crop Science Division, Germany, (5)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (6)RIFCON GmbH, Germany, (7)ibacon GmbH, Germany, (8)knoell France SAS, France, (9)HSE, United Kingdom, (10)Swedish Chemicals Agency, Sweden, (11)German Environment Agency (UBA), Germany*

In the last years, mechanistic effect models (MEMs) have been increasingly developed and proposed for application in the environmental risk assessments of plant protection products. The MAD book (Model Acceptability and Scenario Development) is the product of a multi-partite working group of the SETAC Europe Interest Group on Mechanistic Effect Models with the aim to provide some means to adequately assess mechanistic effect models for their use for the environmental risk assessment of chemicals, here specifically of pesticides. In this poster we summarize chapter 1, giving an overview on realized and potential uses within the regulatory framework of the European Union.

MEMs have been used to dynamically simulate effects on organisms, populations, communities and ecosystems. So far, they have been proposed mainly in a number of predictive ways as refinement options within the tiered approach. However, MEMs may be also used in a retrospective way to improve the analysis of ecotoxicological data and to understand the margin of safety associated with established assessment factors. MEMs may be more complex than currently used methods. Still they may increase our understanding of the level of uncertainty associated with predictions of environmental effects. However, MEMs stand and fall with an understanding and appropriate implementation of relevant mechanisms. Therefore, dynamics and pesticide effects predicted by MEM simulations are required to be validated, i.e. tested with independent data or knowledge. The question of how (e.g. at which scales and with which precision) this can be done remains a challenge, especially for large scale ecological models or models for vertebrates.

The EFSA Scientific Opinion on Good Modeling Practice (2014) and protocols such as TRACE and ODD are supportive for the development, application and assessment of MEMs in regulatory risk assessment. The MAD book provides modelers and risk assessors with recommendations to develop the necessary explicit quality criteria for the various aspects of the modeling process, including the underlying data, the conceptualization, implementation, the environmental scenario, model validation, the model performance and the documentation.

#### **4.04.P-We299 The MAD book - Development of Environmental Scenarios for the Application of Mechanistic Effect Models in Environmental Risk Assessment**

*Sanne van den Berg<sup>1</sup>, Sabine Duquesne<sup>2</sup>, Oliver Jakoby<sup>3</sup>, Udo Hommen<sup>4</sup>, Thomas G Preuss<sup>5</sup>, Magnus Wang<sup>6</sup>, Alpar Barsi<sup>7</sup>, Stefan Reichenberger<sup>8</sup> and Andreas Focks<sup>9</sup>, (1)Wageningen University & Research (WUR), Netherlands, (2)German Environment Agency (UBA), Germany, (3)RIFCON GmbH, Germany, (4)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (5)Bayer AG - Crop Science Division, Germany, (6)WSC Scientific GmbH, Germany, (7)Board for the Authorisation of Plant Protection Products and Biocides (Ctgb), Netherlands, (8)knoell France SAS, France, (9)Institute of Mathematics, Osnabrück University, Germany*

Mechanistic effect models (MEMs) offer a comprehensive platform for analyzing the environmental risk of pesticides across various biological, spatial, and temporal scales. In essence, MEMs make predictions about the environmental effects of pesticides or other stressors based on mechanistic principles embedded in the model. These principles derive from physical, chemical, and biological rules, often influenced by environmental conditions such as temperature, pH, and food availability. Environmental scenarios, defined as combinations of abiotic, biotic, agronomic, and sometimes landscape conditions, are

crucial when utilizing MEMs for meaningful assessments. The differentiation between ecological and exposure scenarios is made, with certain factors belonging exclusively to one or the other, and some (primarily abiotic factors) encompassing both. The dual influence of certain factors on exposure and ecological scenarios underscores the importance of consistent environmental scenario definitions. The lack of standardization in defining environmental scenarios, coupled with a dearth of guidance on formulating and evaluating such scenarios in model assessments, is addressed in this presentation of a chapter of the MAD book. We review historical approaches to defining exposure, ecological, and environmental scenarios in environmental risk assessment, offering a vision for future definitions and evaluations. To aid modelers and risk assessors, we propose a catalog of questions, designed to establish well-founded, comprehensive, and structured environmental scenarios for MEM-based risk assessments. Finally, three examples are given how environmental scenarios can be defined in MEM-based chemical risk assessments, accompanied by a brief discussion of each. This comprehensive overview and proposal provide valuable insights, setting the stage for future advancements in environmental scenario definition and evaluation within the context of MEM applications

#### **4.04.P-We300 The MAD book - Documentation and Evaluation of Data Used in Mechanistic Effect Models**

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Pesticide regulation in the European Union requires a robust evidential basis to underpin decision-making. When an applicant relies on a mechanistic effect model to demonstrate that the use of a plant protection substance will not lead to unacceptable effects, it is crucial to clearly illustrate how the model has been utilised to address specific risk assessment questions. The utilisation of available empirical data in addressing these questions is equally important, as the quality and appropriateness of the input data significantly impact the model outputs. Consequently, regulatory risk assessors need to scrutinise the underlying studies providing the data used in the model implementation. Given the relatively novel use of mechanistic effect models in pesticide risk assessment, transparent documentation of the quality and appropriateness of input data is of particular significance. This documentation must demonstrate the suitability of a mechanistic model for risk assessment to regulators who may be less familiar with this approach. Clarity and transparency in presenting the development and utilisation of a model in risk assessment will enhance confidence in its suitability, establish a solid foundation for future applications, and promote greater adoption of mechanistic models. In general, the data used in the model should align with the regulatory problem formulation for which they are employed, particularly in the context of higher tier risk assessment questions. This necessitates a detailed description of the relevant data used for specific parameters or aspects of the model, clarifying their purpose and the derivation of parameters from the study/studies, including any data transformations. Currently, mechanistic effect models are primarily used as part of a higher tier modelling approach in pesticide risk assessment. As such, this chapter covers documenting and evaluating the use of data for the modelling for environmental risk assessment of pesticides.

#### **4.04.P-We301 The MAD book – Evaluation of modular models in ERA**

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Mechanistic effect models (MEMs) are increasingly considered to be useful tools for ecological risk assessment (ERA) of chemical stressors, amongst others, as they can predict population dynamics of non-target species under toxic stress for different environmental scenarios. So far, the evaluation of complex models that consist of several sub-models or modules for ERA purposes has largely ignored strengths and weaknesses of individual sub-models, modules or specific model parts.

This MAD book chapter aims to introduce the concept of modular model evaluation for the specific case of complex MEMs and its effective implementation in risk assessment. We will highlight the advantages and benefits of considering modularity for the evaluation of the model structure, of parametrisation, and also for validation (including the domain of applicability).

Dividing complex, modular models (e.g., coupled population or ecosystem models including TKTD models) into meaningful and clearly defined modules allows for their individual documentation, testing, evaluation and assessment. Such modular consideration of MEMs would simplify communication about complex models, reduce the workload for model development and evaluation, and allow better differentiation between robust and uncertain parts of the models. In addition, modularisation can help to overcome challenges related to the over-parameterisation of complex models through partial and independent parameterisation and validation of modules.

Questions about transferability to other environmental scenarios and applications could be answered more precisely: Which modules are already sufficiently validated and can be reused in other models? Can already documented and accepted modules be combined in new models, or replaced by simpler or more complex alternatives? These and other considerations highlight the importance of designing modules and system architecture using robust software engineering principles, with clearly defined and consistent interfaces between modules.

From these considerations, a decision scheme was derived that could be used for the validation of modular models for specific risk assessment questions.

#### **4.04.P-We302 The MAD Book - On the Evaluation of Calibration and Validation Outputs with Mechanistic Effect Models**

*Simon Ewald Hansul<sup>1</sup>, Peter Vermeiren<sup>2</sup>, Evgenia Chaideftou<sup>3,4</sup>, Michail Gioutlakis<sup>2</sup>, Udo Hommen<sup>5</sup>, Mira Kattwinkel<sup>6</sup>, Judith Klein<sup>5</sup>, Stefan Reichenberger<sup>7</sup>, Pernille Thorbek<sup>8</sup>, Johannes Witt<sup>9</sup> and Sandrine Charles, PhD, HDR<sup>10</sup>, (1)Institute of Mathematics, Osnabrück University, Germany, (2)RIFCON GmbH, Germany, (3)Benaki Phytopathological Institute, Greece, (4)RECETOX, Faculty of Science, Masaryk University, Czech Republic, (5)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (6)University of Kaiserslautern-Landau (RPTU), Germany, (7)knoell France SAS, France, (8)Apd/EE, BASF plc, United Kingdom, (9)Bayer AG - Crop Science Division, Germany, (10)Umr Cnrs 5558, University Claude Bernard Lyon 1, France*

Before mechanistic effect models (MEMs) can be applied to support environmental risk assessment, rigorous checks of model outputs are critical to evaluate the goodness-of-fit and the model suitability for further predictions. In this book chapter, we prioritized a collection of qualitative, visual assessments as well as quantitative statistics and metrics to support model output evaluation when MEMs are calibrated and validated on experimental or observed data. These criteria are reviewed for their use and limitations. Guidance is given regarding key issues to consider during the evaluation process, how they relate to the methods used, and how the methods can be integrated into a general approach. MEM outputs are of inherent complexity, including multiple state variables, outputs at different levels of biological organization, and a diversity of output patterns and types. We therefore argue for a multi-criteria approach, where methods should be combined to ensure a robust and balanced assessment of the quality of model outputs in reference to both data used for the calibration step and the external data to which predictions are compared. Note that also these data may be diverse and of different quality and types. We further highlight the non-binary, gradual outcome of model evaluation, assigning models a degree of validity with respect to the area of application, rather than a generic judgement about validity/invalidity. While there is not a one-size-fits-all solution to MEM calibration and validation, our evaluation approach should help model developers in choosing the right methods for the different tasks, while also supporting model users and assessors to follow the reasoning behind the methods applied so that they can evaluate and interpret them in the context of evaluating the output of calibration and validation with MEMs.

#### **4.04.P-We303 The MAD Book - Sensitivity and Uncertainty of Mechanistic Effect Models**

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The application of mechanistic effect models (MEMs) to risk assessment is inherently associated with uncertainty. The accurate characterization of this uncertainty, its magnitude, direction, and sources, is a critical step in the utilization of MEMs to support decision making for risk assessment. The uncertainty of MEM output can be quantified by means of uncertainty analysis (UA). Uncertainty in model outputs originates from a variety of sources, including uncertainty in the available data and knowledge, as well as the model structure, parameters, scenario definitions, and the simulation of stochastic processes. Uncertainty propagates throughout stages of the model development process to the application of the model, and thus requires careful attention all along the modelling cycle. UA is tightly linked to sensitivity analysis (SA), which focusses on quantifying the variability in model output relative to the variability in model input factors. In the case of MEMs, the model input factors are mainly the biological and environmental parameters. Performing both UA and SA is part of a comprehensive model development and application process, but they can be performed during different stages of the model development process with slightly varying motivations. We classify sources of uncertainty and provide suggestion for how to account for them in the modelling process. In doing this, we argue for a multicriteria approach, considering uncertainty using different metrics and evaluations from various perspectives. A comprehensive evaluation of MEMs generally requires an approach tailored to the specific MEM aims and objectives. We provide some general guidance on when and how to perform SA and UA and provide a simple example to illustrate the process, using a population model to assess the uncertainty in population-level effects resulting from biological baseline and toxicity parameters. Finally, we provide a concise list of non-technical, high-level considerations in order to aid the evaluation of UA and SA by both model developers and evaluators. A well-balanced, multifaceted UA and SA dramatically increases the usefulness of MEMs for risk assessment by providing a systematic approach to map uncertainty about the knowledge of a system to uncertainty in model predictions, and therefore answers to extrapolation problems in risk assessment.

#### **4.04.P-We304 Use of Toxicokinetic-Toxicodynamic (TKTD) Models in Pesticides Risk Assessment for Aquatic Organisms – Feedback from a National Regulatory Agency**

*Harry Byers, Claire Blondel, Fannie Chouvellon, Emilie Perrat, Pierre-François Chaton, Emilie Farama and Vanessa Mazerolles, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France*

According to the European regulation (EC) No 1107/2009, a regulatory risk assessment (RA) is performed for all Plant Protection Products (PPP). For aquatic organisms when exposure cannot be excluded, a RA, conducted following the EFSA

Guidance Document on Aquatic Ecotoxicology (2013), is required. This document anticipated the use of TKTD models for risk assessment. The EFSA TKTD scientific opinion, released in 2018, covers the state-of-the-art of TKTD models for aquatic organisms and provide recommendations for their use within the EU's regulatory framework for plant protection products.

This scientific opinion focuses on prospective environmental RA and proposes TKTD models as a refinement option (Tier-2C) in acute and chronic RA schemes, aligned with EFSA PPR Panel approaches as described in the EFSA Guidance Document on Aquatic Ecotoxicology (2013). The opinion provides guidance on TKTD model application in RA and outlines specific validation steps for inclusion in the process.

Since the publication of the TKTD opinion 2018, around 25% of Active Substance (re)approval dossiers received by ANSES, acting as Rapporteur Member State, integrate TKTD models, notably GUTS and *Lemma* models, for risk assessment refinement. Despite their prevalence, challenges and advantages in practical application have emerged.

The assessment of these models supports the initial argumentation favouring TKTD models in risk assessment, as outlined by the EFSA opinion (2018). A key advantage lies in considering the complete aquatic exposure profile of the studied chemical, either for the toxicity studies or for the Predicted Environmental Concentration profile used in the risk assessment. However, challenges persist. Although the 2018 opinion streamlines model assessment, the evaluation process for TKTD models remains time-consuming due partly to inconsistencies in interpreting opinion recommendations. This necessitates additional time for standardization efforts.

The objective of this poster will be to present the view of a national regulatory agency on the use of TKTD models in aquatic risk assessment. The poster will provide feedback from regulatory risk assessors on the advantages and limitations of using these models in a regulatory framework for predicting the risk of chemicals to aquatic organisms. Technical and practical aspects will be tackled. The poster will also touch upon potential areas for improvement in this field.

#### **4.04.P-We305 New perspectives on the body burden model for bird risk assessment of pesticides**

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Introduced in 2016 as a refinement option for vertebrate environmental risk assessment, the body burden model (BBM) was initially not very popular, and very little use was made of it either in the scientific literature or for regulatory purpose.

However, the BBM is a simple toxicokinetic model that easily allows the description of the internal dose through time when individuals are exposed to contaminated food via different feeding patterns. The BBM is considered as a possible option by in its most recent updated guidance on risk assessment for birds and mammals. This talk proposes a fully revisited BBM, in terms of mathematical writing, computer implementation and simulation interface. After a quick overview of the founding equations and the web interface, two case studies will be shown to illustrate the operative use of the BBM for regulatory purposes.

#### **4.04.P-We306 Effect of Time Variable Concentrations on Green Microalgae: Evaluating the Robustness of a Laboratory Comparison Test using Toxicokinetic-Toxicodynamic Modelling**

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Toxicokinetic-Toxicodynamic (TKTD) modelling has become an increasingly recognized option in risk assessment. For algae, the lack of a standardised and ring tested test setup for time variable exposure has so far been the major drawback for the application of TKTD modelling, according to the European Food Safety Authority's (EFSA) opinion on TKTD modelling for use in risk assessment.

A laboratory comparison test has therefore been organised by CropLife Europe, to evaluate the robustness and reproducibility of two methodologies, a semi-static and a flow-through setup, for generating data for time-variable exposure on green microalgae. Traditional ring test statistics have been used to demonstrate the robustness of the semi-static method.

However, due to the time-variable exposure pattern, selection of appropriate robustness criteria is not straightforward. Amongst others, it is unclear which statistics should be used to compare time-variable exposure with constant exposure (i.e. in a standard algae study).

We therefore additionally evaluate the robustness of the semi-static test design using TKTD modelling. For this aim, we calibrate an algae TKTD model separately to each test run in each of the laboratories. Each of these calibrated models is then used to predict *in silico* the ErC<sub>50</sub> of a standard OECD 201 algae test.

The resulting set of ErC<sub>50</sub> values allows for a straightforward comparison of the results to standard algae studies.

#### 4.04.P-We307 Global Sensitivity Analysis of the Harmonised Lemna Model

*Chloé Guisnet<sup>1</sup>, Stefan Reichenberger<sup>1</sup> and Frank Voss<sup>2</sup>, (1)knoell France SAS, France, (2)knoell Germany GmbH, Germany*  
Ecological and effect modelling is becoming more and more important for environmental risk assessment in the framework of pesticide authorization. For instance, the EFSA Scientific Opinion on TKTD modelling has judged the GUTS model for lethal effects and the model for the standard aquatic macrophyte test organism Lemna as “ready for use”. Nevertheless, national regulatory authorities are still hesitant to accept ecological and effect modelling studies because they do not have sufficient experience with these models.

One way to increase the confidence in a model is to perform a Global Sensitivity Analysis (GSA) to analyze the contribution of the different model input factors to the variance of the model output(s).

In the case of ecological and effect models, a GSA notably allows to identify and rank the importance of i) toxicokinetic and toxicodynamic parameters, ii) physiological and ecological parameters of the organism, and iii) environmental driving variables (e.g. radiation, temperature, nutrient concentrations).

In this study we performed a two-step global sensitivity analysis (GSA) of the refined and harmonized Lemna model, which is publicly available as an R package. In a first step a Morris sensitivity screening was conducted. This semi-quantitative global method is very parsimonious and allows to filter out non-influential input factors. In a second step, a true variance-based GSA was carried out with the Sobol’ method and a reduced set of input factors. The GSA was conducted for four different concentration levels and three different exposure regimes: i) constant exposure, ii) two exposure pulses with varying intervals between peaks, and iii) realistic exposure time series generated with the FOCUS surface water models. Moreover, two different sets of input distributions of TKTD parameters were examined: i) distributions reflecting the parameter range for a specific substance (example: metsulfuron-methyl); ii) distributions reflecting the whole realistic parameter range (different substances). The main target variables were the effects of the pesticide on biomass and on growth rate.

Both Morris and Sobol GSA showed that for a specific substance three physiological parameters and the initial biomass BM0 were more important than the TKTD parameters. Hence, for predictive applications of the model outside a laboratory context, BM0 must be chosen carefully, and uncertainty in the main physiological parameters must be reduced to a minimum.

#### 4.04.P-We308 Predicting Effects of Time-Variable Exposure Concentrations on Primary Producers using the *cvasi* Software Tool

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Toxicokinetic-Toxicodynamic (TKTD) modelling is increasingly used in European regulatory risk assessments of plant protection products, following the publication of EFSA’s scientific opinion on TKTD modeling. This creates a demand for software that allows quickly and smoothly going through the whole workflow required for regulatory submissions: from model calibration and validation, over effect calculation for exposure scenarios, up to calculation of the respective regulatory endpoints such as LP<sub>50</sub> and EP<sub>50</sub> values.

A first version of our software tool ‘*cvasi*’ (calibration, validation, and simulation of TKTD models in R) is planned to be released before SETAC Europe as open source. The tool enables prediction of time-variable exposure concentrations on primary producers, use of models for algae, *Lemna*, and *Myriophyllum* at Tier 2C. It includes a user friendly and interactive R-Shiny interface, as well as routines for plotting and calculation of EP50 values, based on the whole profile or on a moving time window.

In future versions of the tool, we intend to include functionalities for calibration and validation, calculation of confidence intervals using likelihood profiling, and mixture toxicity. The structure of the tool also allows for integration of other TKTD models, such as GUTS and DEB, or even custom models, as the tool develops in capabilities.

To demonstrate the tool, we predict the effects of time-variable exposure concentrations on primary producers (algae, *Lemna*, and *Myriophyllum*) for a set of example substances.

#### 4.04.P-We309 Toxicokinetic-Toxicodynamic Modelling Application (for Lemna sp.) in Regulatory Risk Assessment Scheme: Conceptual Stepwise Approach

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Toxicokinetic-toxicodynamic (TKTD) models are becoming increasingly reliable in approximating the effects of a chemical in organisms by considering time-varying exposure. Its use for the risk assessment of Plant Protection Products (PPPs) under Regulation 1107/2009 is an acceptable approach according to the regulatory guidelines currently in force. Indeed, in 2018, EFSA published a scientific opinion stating that some of these models, such as the *Lemna* model, can be considered “ready to use”. In addition, EFSA has recently made available a new platform for TKTD modelling of chemicals to apply new methodologies for approaching chemical risk assessment. In practice, however, regulatory acceptance of such models is

uncertain. The lack of a reference case or a clear scheme on how these models should be used to support the risk assessment of PPPs hampers the acceptance of these modeling approaches. In this presentation, we describe the stepwise application of a TKTD model for *Lemna* in the context of a risk assessment for a hypothetical herbicide, taking into account the standard ecotoxicological data packages that are required for the European authorization of PPPs. Firstly, we detail the assumptions needed to consider the applicability of these models for covering all sensitive species in the assessment. Secondly, we explicitly describe the minimum data that should be available for validation and calibration of the model and the additional ecotoxicological data that could be used as supplementary information. This is followed by a description of the various steps that need to be considered in order to apply the model, together with the potential challenges associated with each of them. As a result, a conceptual stepwise approach is presented by identifying the ways in which the information provided by the TKTD model can be used within the risk assessment procedure. In conclusion, this work provides a practical example of the applicability of a TKTD model for *Lemna* in the different steps of the risk assessment scheme, highlighting its use to reduce the uncertainty associated to the rest of available ecotoxicological data. This hypothetical case could therefore be seen as a 'blueprint' of how TKTD modelling can be used to support the decision-making process for PPPs.

#### **4.04.P-We310 Using Mechanistic Modelling to Understand Species Sensitivity to Xenobiotics: A Case Study in Birds**

David Heckmann<sup>1</sup>, Vanessa Baier<sup>2</sup>, Markus Ebeling<sup>3</sup>, André Gergs<sup>1</sup>, **Thomas G Preuss**<sup>1</sup>, Pavel Balazki<sup>2</sup> and Stephan Schaller<sup>2</sup>, (1)Bayer AG - Crop Science Division, Germany, (2)esqLABS GmbH, Germany, (3)Environmental Safety, Bayer AG, Germany  
Environmental risk assessment for xenobiotic substances needs to account for effects on a plethora of wild species, but effect data is usually only available for a small number of model species. Since an increase in animal testing is generally not desired and might be unfeasible, mechanistic toxicokinetic toxicodynamic (TKTD) modelling can be used to assess species-specific risk by integrating knowledge on species physiology and/or species-specific *in vitro* data. Nevertheless, differences in species sensitivity are rarely understood mechanistically, making the required datasets for species extrapolation unclear and limiting the confidence in model-based extrapolation approaches. There is thus a need for case studies that mechanistically elucidate differences in species sensitivity.

For the fungicide and nematicide fluopyram, bird reproduction studies resulted in notably lower reproductive effect concentrations in the bobwhite quail (*Colinus virginianus*) compared to the mallard duck (*Anas platyrhynchos*). In order to understand differences in reproductive endpoints between the two avian species, we developed physiologically based kinetic (PBK) models of the compound in chicken, bobwhite quail and mallard duck. These models were based on the previously developed generic models for the three species in the Open-Systems-Pharmacology Suite (OSP Suite with PK-Sim® and MoBi®) and used available chicken *in vivo* data and species-specific *in vitro* data to capture fluopyram toxicokinetics. The predictions of the PBK model for the quail have been previously combined with a dynamic energy budget (DEB) TKTD model of embryo and hatchling growth to successfully predict the endpoints of the quail reproduction study. We now used the duck fluopyram PBK model to simulate the mallard duck reproduction study. The model predicted notably lower concentrations of the benzamide metabolite in eggs of mallard ducks compared to bobwhite quail, suggesting that differences in fluopyram toxicokinetics could explain the observed lower sensitivity of the mallard duck. The model set-up can now be used to mechanistically analyze the physiological factors that underly the differences in species sensitivity to fluopyram, thus providing a case study for the mechanistic elucidation of species sensitivity.

#### **4.04.P-We311 A Dynamic Energy Budget Model of Anuran Larval Development for Environmental Risk Assessment.**

Carlo Romoli<sup>1</sup>, Erik B Muller<sup>1</sup>, Douglas J. Fort<sup>2</sup>, **Benoit Goussen**<sup>1</sup>, Lennart Weltje, Prof. Dr.<sup>3</sup>, Joachim Ulrich Kleinmann<sup>4</sup> and Pernille Thorbek<sup>5</sup>, (1)ibacon GmbH, Germany, (2)Fort Environmental Laboratories, Inc., (3)Agricultural Solutions - Ecotoxicology, BASF SE, Germany, (4)BASF SE, Germany, (5)Apd/EE, BASF plc, United Kingdom

The metamorphosis of anuran amphibians is a plastic process that can be influenced by environmental conditions and/or toxicant exposure. This is considered to be a strategy adopted to increase the chances of survival. In a favorable environment, development can be slower and a more advantageous larger size at metamorphosis can be attained. In unfavorable environments, the development is accelerated, even at the cost of a smaller size after metamorphosis, in order to escape lethal conditions, such as the drying out of a pond or a high predation pressure.

A mechanistic model that would take into account the plasticity of metamorphosis would aid the assessment of endocrine disruptor compounds (EDC), among other toxicants. Such a model can provide a mean of comparison between the toxic effects on the development, as detected from regulatory amphibian tests (OECD TG 231, 241, 248), and the natural variability of the metamorphosis process due to the environmental factors.

We developed a Dynamic Energy Budget model that includes details of the metamorphosis process, as well as its response to environmental conditions. We focus here on temperature, a well-studied factor that has the double effect of accelerating biological rates, and to be a natural stressor that in several species can accelerate metamorphosis at the expense of final froglet/toadlet size.

We evaluate model performance with published data on the species widely used in laboratory testing (e.g., *Xenopus laevis*, *Rana temporaria*) and with data on *Dryophytes versicolor*, which were generated specifically for this project.

#### **4.04.P-We312 Comparative Energetics and Toxicant Response of Standard Laboratory Fish Species and European Edge of Field Species – Are We Really Protective?**

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Standard fish toxicity tests are conducted with a limited number of species chosen for standardization, cost and convenience reasons. The extent to which those laboratory species are representative for other fish species remains a major open question in Environmental Risk Assessment (ERA). We investigate how bioenergetic properties of European edge-of-field (EoF) species compare to those of standard laboratory fish (SLF) species and evaluate the implications those (dis)similarities may have on expected impacts on common endpoints due to toxicant exposure. For this purpose, we use the publicly available Add-my-Pet (AmP) collection of Dynamic Energy Budget (DEB) parameters and derived traits. We identified 10 standard laboratory species and 20 EoF species with widespread distribution in the EU with representation in the AmP collection. Eco-physiological properties of EoF species are generally in line with those of ray-finned fish of similar size, while maintenance demands and assimilation capacities of SLF species tend to be high. This implies that, among other things, that SLF tend to grow relatively fast to their maximum size. In addition, SLF tend to allocate a relatively large fraction of their resources to reproduction. Those properties contribute to their suitability as laboratory species. We explored the implications of toxicant exposure by assuming physiological modes of action (PMoA) on DEB parameters, including those indicative for endocrine disruptive action, of equal strength for all species. The results show a mixed bag, with expected relative changes in endpoint depending on species, PMoA and choice of endpoint, as will be presented in detail during the meeting. This study is an example demonstrating the utility of the AmP collection and associated software applications for ERA purposes.

#### **4.04.P-We313 Comparative Energetics for Environmental Risk Assessment of North American Birds**

*Erik B Muller<sup>1</sup>, Carlo Romoli<sup>1</sup>, Benoît Goussen<sup>1</sup>, Jonathan D. Maul<sup>2</sup>, Richard Brain<sup>2</sup> and Nika Galic<sup>3</sup>, (1)ibacon GmbH, Germany, (2)Syngenta Crop Protection, (3)Syngenta AG, Switzerland*

A major challenge in Environmental Risk Assessment (ERA) is to evaluate the implications of data from standard toxicity tests with a limited number of species in the context of species diversity. The question, then, is if and how we can combine the extensive ecotoxicological knowledge we have for a limited number of species with the little we know about the majority of species, including vulnerable and endangered species. Additionally, identifying focal species and species groups with field relevance is further challenged by data scarcity, which also hampers the development of population models for risk assessment. To address this challenge, we compare eco-physiological traits across avian species, specifically of test species with species of ERA interest and terrestrial Holarctic birds as a whole. These traits are drawn from the Add-my-Pet (AmP) collection, a data base of Dynamic Energy Budget (DEB) parameters and derived trait values of more than 4,000 animals, including 446 terrestrial Holarctic birds and 72 species of ERA interest. Reproductive abilities of standard lab species were high compared to other birds of similar size. However, traits of other terrestrial-focused species and waterfowl typically were in agreement with other birds of similar size, suggesting the important role strain selection in and experimental design of standard toxicity tests have on data collected for DEB model parameterization. Multidimensional scaling analysis of parameter and trait values of Holarctic birds in the AmP collection shows clustering of major taxonomic avian groups. Clustering of species of ERA interest grouped according to their feeding guild during the breeding season appeared weak, with most of the clustering reflecting taxonomic identity rather than guild membership. This study is an example demonstrating the utility of the AmP collection and associated software applications for ERA purposes.

#### **4.04.P-We314 DEB-TKTD analysis of avian reproduction studies: overview of two case studies**

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Mechanistic modelling to analyse the effect of chronic exposure of chemicals to birds is rapidly gaining interest in the environmental risk assessment field. The new EFSA Guidance document on risk assessment for birds and mammal includes the possibility to use toxicokinetic-toxicodynamic (TKTD) models. In particular, the use of a TKTD module in combination with a physiological model like the Dynamic Energy Budget (DEB) model is recommended. However, examples of case studies using DEB-TKTD models to analyse toxicity test data for birds are currently lacking. Here we present two recently developed DEB-TKTD models to analyse avian reproduction studies. While both studies implemented their own adaptation of the standard DEB model to the specificities of bird physiology and avian reproduction studies, they differ by their predicted endpoints and their level of complexity. In the first study, food ingestion data as model input was used in order to account for the observed up-regulation of feeding during egg-laying. This also allows disentangling and quantifying possible behavioural effects from direct toxic action of the pesticides. The second study offers a deeper mechanistic approach of the feeding upregulation phenomenon and also predicts effects on offspring growth but does not include behavioural responses yet. The two models are compared, stressing their strengths but also the possible model improvements needed to enhance their use and their acceptability in environmental risk assessment.

#### **4.04.P-We315 Feeding inhibition : starvation : chronic mortality – a dynamic energy budget approach**

*André Gergs, Bayer AG - Crop Science Division, Germany*

The risk assessment of pesticides makes use of acute (short-term) and chronic (longer-term) toxicity tests. The aquatic assessment typically focusses on mortality for acute and sublethal endpoints for chronic toxicity. Sublethal endpoints include growth, development, and reproduction, but also chronic mortality may play a major role. Toxicokinetic-toxicodynamic (TKTD) models, such as the General Unified Threshold model of Survival (GUTS), allow the extrapolation of endpoints across



different time scales, such as acute and chronic. In cases where underlying toxicity mechanisms are different for acute and chronic effects, the extrapolation potential of GUTS might, however, be limited. One example is prolonged starvation upon chemically induced feeding inhibition which may result in chronic mortality: In this study, a short-term feeding inhibition test with the non-biting midge *Chironomus riparius* and imidacloprid was conducted to quantify sublethal effects. I applied a Dynamic Energy Budget based TK-TD model to analyze the feeding inhibition test and simulate subsequent starvation to explain observed chronic effects.

#### **4.04.P-We316 To DEB or not to DEB – Earthworm Modelling using Standard Ecotox Data**

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The risk assessment of earthworms considers in the lower tier only exposure in the top 5 cm of the soil. However, in natural conditions exposure dramatically varies in space and time which leads to a much more complex exposure pattern. Therefore, modelling options which help to understand how exposure leads to effects are urgently needed.

In the presented case-study we evaluated the data needs of two existing modelling approaches (Dynamic Energy Budget, DEB modelling and Physiological Energy Budget, PEB modelling) for parameterization and validation.

Toxic effects in the widely used DEB models can only be parameterized if data on the development of effects over time is available. Since sampling in earthworm studies is destructive, the production of such data is very labor- and cost expensive and not part of the standard toxicity dataset of earthworms. This means, such a standard toxicity dataset is not sufficient to parameterize DEB models. In the alternative PEB model, energy is not distributed in parallel to somatic and reproductive processes (kappa rule) as in the DEB model but sequentially to maintenance, reproduction, growth and reserve. Therefore, this approach saves several parameters and toxic effects are reflected by a dose-response model.

We were able to parameterize the PEB model for the standard earthworm species in laboratory studies *Eisenia fetida* for three example substances using the control dataset and the toxicity model using the treatment data of the corresponding study. To validate the models, we used product studies (single active and mixture products) of the same active ingredient. In mixture products, the model was able to distribute the relative effects to the two active ingredients. To increase the confidence in the models, also validations against (semi-)field data could be done.

For the presented substances, PEB modelling worked very well without additional tailor-made laboratory studies. We believe that if a PEB model can be calibrated and validated, the model should be acceptable for the risk assessment of the corresponding substance. Since for this model also versions for *Aporrectodea caliginosa* and *Lumbricus terrestris* are available, we think that it can be a useful lower-tier modelling option to refine the earthworm risk assessment even when only standard toxicity data is available.

If validation is not possible for a substance, additional data can be created for DEB modelling as a higher modelling tier.

#### **4.04.P-We317 A Conceptual Approach to Determining Normal Operating Ranges: Outcomes from a Multi-Stakeholder Workshop**

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Normal operating ranges (NOR) are defined as any natural variability in a system property, including population density or abundance. There is an increasing regulatory focus on determining NORs as both a mean to facilitate the design of field studies that can reliably demonstrate a certain effect level, and for consideration in population modelling. However, there are currently no approaches that have been sufficiently developed and tested for the purposes of NOR determinations. Possible approaches include mechanistic models as well as analyses of field data, but they both have advantages and disadvantages. For instance, field data are often incomplete, cover only a subset of environmental contexts, and of limited duration and spatial extent. Mechanistic models can provide predictions across relevant temporal and spatial scales and extrapolate across relevant environmental context. However, whether they are fit for the purpose of NOR determination is not always clear as they are not commonly developed to predict variability realistically. Rather, they aim to predict typical, or worst-case, responses and system properties.

To address this gap and propose a conceptual approach for quantifying NORs for the purposes of ecological risk assessments, CropLife Europe organized a multi-stakeholder workshop. Experts with background in data analysis, mechanistic modelling, and risk assessment were brought together in a 2-day workshop. The workshop consisted of presentations and discussions on the use of models and data – in isolation and in combination – and the advantages and limitations of each were considered. Workshop participants proposed an approach on how current knowledge and state-of-the-art methods can be leveraged and further developed to establish normal operating ranges of focal species and systems that are both comprehensive and operational.

#### 4.04.P-We318 Determining Normal Operating Ranges from Field Data: A Wood Mouse Case Study

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Normal operating ranges (NOR) are defined as any natural variability in a system property, such as population density or abundance. There is an increasing regulatory focus on determining NORs as both a mean to facilitate the design of field studies that can reliably demonstrate a certain effect level, and for consideration in population modelling. Recently, mechanistic effect models have been used to determine NORs, but it is not yet clear if and under which conditions they are fit for this purpose. A complementary approach is to derive NORs based on available data to better understand this variability in the field. Here we present the results of an analysis focusing on a species important for mammal risk assessment - the wood mouse, *Apodemus sylvaticus*. For the analysis, data published in peer-reviewed literature were used in addition to data compiled from generic field studies conducted by agrochemical companies. Data contained information on mice densities in different environmental contexts, including agricultural and more natural habitats. Data were analysed with generalized linear and generalized additive models, as well as with machine learning approaches. Results were interpreted as variability in abundance or densities across relevant habitats and environmental contexts, e.g., NOR of densities across hedge-dominated margins in cereal fields in summer months. Data gaps and challenges were identified. Possible uses and applications of these outcomes for population modelling are discussed, as well as suggestions on how to better integrate existing data with modelling approaches for a comprehensive analysis of NORs of non-target populations in the field.

#### 4.04.P-We319 Ecological modelling in support of sediment remediation: impact of nature-based remediation on *Hyalella azteca* populations

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The EU-LIFE project NARMENA (Nature-based Remediation of Metal pollutants in Nature Areas) aims to evaluate the efficacy of Nature-Based Remediation (NBR) techniques in mitigating metal pollution in soil and sediment within select nature reserves in Flanders, focusing on three target sites. A crucial component of this project involves the development of an ecological modelling tool to assess the positive impact of NBR techniques on the ecological risk within the local area in comparison with alternatives. The ecological modelling aspect is centred on three indicator species in the water column and sediment. This poster presentation focuses on the sediment-dwelling amphipod, *Hyalella azteca*, and its exposure to heavy metals, including cadmium (Cd), arsenic (As), and chromium (Cr).

To evaluate the effects of heavy metal pollution on the amphipod, we used a Dynamic Energy Budget (DEB) based approach. Toxic effects were incorporated based on data from standard laboratory chronic toxicity experiments for Cd, As, and Cr. Additionally, the influence of physicochemical parameters affecting metal bioavailability was considered. Furthermore, we extrapolated these effects to the population level using an individual-based modelling (IBM) approach, with population abundance and biomass as the key endpoints for risk assessment. Models were applied to monitor the effectiveness of non-invasive remediation techniques in three target rivers in Flanders: Grote Laak, Grote Calie, and Winterbeek.

NBR techniques offer promising alternatives to conventional and more disruptive soil remediation methods. This study assessed the impact of different remediation approaches, differentiating between scenarios of no action, conventional techniques (e.g., excavation), and NBR techniques (e.g., phytoremediation and artificial wetlands). Our findings underscore the value of combining laboratory data, in-field measurements, and mechanistic effect modelling to comprehensively evaluate the impact of mitigation strategies in real-world environmental settings. The study will provide insights into the potential of NBR techniques to effectively reduce metal pollution in Flanders' nature reserves while preserving local ecosystems.

#### 4.04.P-We320 Evaluation of Two Different Individual-Based Models to Simulate the Population Dynamics of *Daphnia Magna* in a Long-Term Laboratory Experiment

*Natalie Carmen Dallmann, Monika Hammers-Wirtz, Josef Koch and Tido Strauss, gaiac - Research Institute for Ecosystem Analysis and Assessment, Germany*

In order to generate a corresponding data set that reflects also the fluctuating pattern after the first exponential population growth period, we conducted a 120-day *D. magna* population test under stable temperature and food conditions with two different initial populations (only few neonates versus high abundant and mixed-age start density). This four-month experiment with continuous algae feeding was followed by a starvation phase to determine the time that daphnids can survive by using their energy reserves.

Next, we evaluated the performance of two different types of individual-based models (IBM) for *D. magna* using this long-term data set: The empirical IDamP model, and a new generic implementation of an IBM based on dynamic energy budget theory, termed DEBgen. Both IBMs were embedded in the biogeochemical lake model StoLaM using the same interfaces to environmental and food modules to ensure comparable test conditions.

The comparative model test focused on i) the amplitudes and periodicity of total abundance and age structure under different starting conditions, ii) the synchronisation of reproductive cycles under given feeding regimes, and iii) the representation of survival time under starvation conditions.

In this study, we deliberately use a modular modelling approach in which models can be analysed as modules in an overarching model, allowing comparisons of relatively simple models under more complex and realistic conditions.

#### **4.04.P-We321 Simulating field-realistic effects to *Chironomus riparius* populations from exposure to insecticides: the role of density dependence and food availability**

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Population models combined with toxicokinetic-toxicodynamic (TKTD) effect models are powerful tools for predicting population dynamics of species under different and field-realistic environmental scenarios. As they can consider natural and anthropogenic stressors, they are increasingly used in ecological risk assessment. Such models should include important regulating processes for population dynamics such as density-dependent effects through intraspecific competition for food or cannibalism, which in turn depend strongly on ecological scenarios.

To study the role of density-dependence and food availability in the sensitivity of *Chironomus* populations to insecticides, we used a model for *Chironomus riparius* which combines an individual-based model (IBM), based on physiological dynamic energy budget (DEB) modules and complemented by TKTD effect models for lethal and sublethal processes, driven by dynamic environmental conditions. The core of the model is an individual DEB-TKTD model that has been parametrised for a systemic neonicotinoid insecticide using laboratory tests at different temperatures as well as experimental results on density dependence.

In a comprehensive experimental laboratory study, we found that *Chironomus riparius* experiences a food-dependent development rate, but also a density-dependent mortality rate of more than 60 % at high larval densities, which is probably due to cannibalism. In principle, at high population densities, lethal effects from exposure to the insecticide can be partially compensated by a reduction in density-dependent mortality. Conversely, populations with a low larval density can be assumed to be more sensitive to insecticides. In this simulation study, we analyse and discuss the effects of varying larval densities and food conditions on the sensitivity of populations of *Chironomus riparius* exposed to different insecticide levels under field-realistic conditions.

#### **4.04.P-We322 Challenges for estimating exposure experienced by moving organisms: a movement modelling concept**

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Ecological risk assessments of plant protection products assess both exposure and effects. Much effort has gone into improving the modelling of exposure and effects. However, the location of the individual relative to the area of application largely controls the exposure of the individual, especially in terrestrial habitats, and movement controls location. For many species movement means that exposure is not static, but highly heterogeneous over time. While recommendations for good modelling practice have been issued for TKTD models, population models, and mechanistic effect models in general, the development of movement modules is often ad hoc. Here, we present a review of movement in population models used for ERA and summarise a Workshop ‘Towards unified principles for movement modelling’ held at ECEM2023, where a general framework for movement modelling was discussed and outlined.

The literature review showed that seven factors (Direction, Speed, Motivation, Energetics, Memory, Response to landscape features, Response to other organisms) cover all movement processes included in the models. However, not all models included all factors and there was a great deal of diversity in how complex movement was represented. The models ranged from no movement to correlated random walks and further to sophisticated models including responses to landscape elements and other organisms. Overall, models for terrestrial species were more complex than aquatic species and vertebrate models were more complex than invertebrates. This diversity to some extent reflects the ecology of the species modelled, and the role movement plays in exposure assessment. Nevertheless, we found that justification for the complexity (or lack thereof) was often lacking or sparse.

The workshop at ECEM2023 was attended by ecological modellers with a broad range of expertise. The workshop took the form of a brainstorm followed by a discussion about the broad outline of a framework. Based on the discussions we formulated a preliminary universal framework for movement modelling, which will be presented. A universal movement modelling framework will help the development and assessment of models. It is not the intention that all movement models must include all factors, but rather that it will help the modellers make informed decisions about what to include and what to exclude. When factors are excluded, justification should be given for why the factors are not considered to be important.

#### **4.04.P-We323 Quantification of Risk from Pesticide Exposure in Landscape-scale Population Models**

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The risk that pesticide exposure on landscape scale poses to organisms can be assessed using spatially-explicit models of the dynamics of the species in the landscape. In a landscape-level approach protection goals relevant to the spatial population need to be defined, as well as the endpoints and specific metrics required to evaluate whether goals are reached. In this presentation we explore such endpoints and metrics, specific to the landscape scale.

Simulations were run with an individual-based population model for aquatic macro-invertebrate, *Asellus aquaticus*. The model contained Dynamic Energy Budget and toxico-kinetics toxico-dynamics (GUTS) submodels for individual growth and development, and for impact of the pesticide on daily survival. Simulated exposure was obtained from the Rummen catchment case-study.

Elements of the modelling and risk assessment approach include 1) a meta-population representation of the spatial population structure, assuming that each reach in the catchment potentially supports a local population, 2) ecological threshold approach, focussing on population dynamics over multiple years with exposure, 3) a margin-of-safety approach, evaluating effects under a range of concentration multiplication factors.

Impact can be quantified at different spatial and/or ecological aggregation levels, and analysed in non-spatial, spatially-implicit or spatially-explicit ways. We test metrics of the total (meta)population and the number of non-extinct local populations. In more spatially explicit metrics catchment branches were defined and population characteristics per branch were analysed. The structure of the functional ecological network was analysed defining groups of functionally connected and persisting local populations.

Total metapopulation size and average number of occupied (non-extinct) local populations are easily observed metrics. Maps of ecological networks indicate the areas where the catchment populations are least threatened. Maps of average local (reach) population characteristics, aggregated into branches, provide insight into how local populations perform.

In the presentation, the pros and cons of approaches to aggregation will be discussed.

#### **4.04.P-We324 Exposure and Effect Modelling for Aquatic Primary Producers at Catchment-scale**

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Risk assessment for aquatic primary producers due to pesticide entries into surface water bodies is conducted in a tiered approach (EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters, 2013). At higher-tiers, more realistic exposure modelling is combined with effect models using real-world environmental and agricultural data. This level of realism allows to quantify effects and their related spatial and temporal extents as required by the definition of Specific Protection Goals.

A catchment-scale model has been developed for this purpose (<https://github.com/xlandscape/xAquaticRisk>). The underlying xAquatic framework has a modular design. When used in a regulatory risk context, components (i.e., modules) are typically built from models which are accepted in the regulatory scientific environment. We employed components already existing in xAquatic on pesticide use, drift exposure and aquatic efate together with newly developed ones on drainage and runoff exposure, to model herbicide entries into streams and their transport and fate in the stream network. Components to model effects of aquatic primary producers (for algae, Lemna and Myriophyllum) were integrated into the catchment-scale model xAquatic. To the user, xAquatic appears as one model with one parameterisation. The spatiotemporally explicit outcome on exposure and effects is stored in a multidimensional storage and can be analysed using common tools (e.g., R, Python, Knime). A set of standard tools on data analysis and reporting are being developed (Jupyter notebooks) which provide modelling endpoints that can be directly compared to defined Specific Protection Goals for aquatic primary producers.

The xAquatic model was applied to an agricultural catchment 'Grote Kemmelbeek' in Belgium. This catchment was subject to an intense monitoring campaign on herbicide residues in the stream network. The connection of modelling and monitoring is expected to provide new insights into agricultural (eco)system behaviour with respect to pesticide use induced exposure and effect levels to primary producers and their spatiotemporal extents, as well as the importance of risk mitigation effects.

#### **4.04.P-We325 Consistent wildlife modelling across Tiers of the European environmental risk assessment**

*Dirk Nickisch, Alexander Singer, Thomas Martin and Jan-Dieter Ludwigs, RIFCON GmbH, Germany*

According to new EFSA wildlife guidance for environmental risk assessments (ERA) of pesticides, the toxicological effects of a substance on the wild rabbit (*Oryctolagus cuniculus*) must be considered as standard scenario. Following the guidance, toxicodynamic-toxicokinetic (TKTD) models have been proposed as a refinement at Tier 2 of the effect assessment and as highest tier, population modelling is considered a possible refinement option.

A population model should be consistent with the modelling of TKTD-processes at Tier 2. Consistency can be achieved by incorporating the effects on individual reproduction predicted by the TKTD model into the population model to assess potential population level effects or recovery.

The wild rabbit has a great reproductive potential, and several litters per female and year are common. On the other hand, mortality within the first year can be as high as 90%. Therefore, modelling the effects on growth and reproduction using a DEB-TKTD for many individuals could provide information on whether high reproduction could buffer toxicological effects or exacerbate the effects, e.g. by carryover toxicity.

Our DEB-TKTD-IBM for the wild rabbit can be used as a first step of the higher tier effect assessment of ERAs. It is integrated in a modular software environment, which allows modelling the individual's life cycle. The model follows assumptions and outcomes of lower Tier risk assessment, such as foraging patterns. This ensures consistence among Tiers.

Commonly, population models for application at higher tier consider different environmental and behavioural complexities and their interactions, such as resulting from animal movement and dispersal. Constructing such models requires substantial effort. Model development usually takes years. Further, the complexity often impedes consistency with lower Tier assessments. In contrast, our spatially implicit modelling approach is readily available and based on the underlying risk assessment Tiers. If necessary, its modular approach even allows step-wise additions of additional processes.

#### **4.04.P-We326 Mapping the Watershed: A Landscape Modeling Approach for Higher Tier Risk Assessment of Lemna in Surface Waters**

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Landscape-scale modelling of hydrology, chemical fate and exposure and resulting ecological effects represent the highest tier to refine the surface water risk assessment for plant protection products (PPP) as mentioned in the Aquatic Guidance Document. Based on local environmental and agronomical information, it can support stewardship activities and the contextualisation of the increasing abundance of public monitoring data. However, regulatory guidance on model or data selection is not in place and further research is needed to evaluate applicability and reliability in a regulatory context.

The present study aims to provide an additional environmental catchment scenario for usage with the recently published open-source model xAquaticRisk (<https://github.com/xlandscape/xAquaticRisk>). The modular model integrates other baseline catchment-scale scenarios and several accepted environmental fate and ecological effect models to run landscape-scale modelling experiments. For the present study, a new scenario was set up for a meso-scale agricultural catchment (54 km<sup>2</sup>) in North Rhine-Westphalia, Germany. The catchment is characterized by a high fraction of arable areas (>50%) and strong variability in rainfall (500-1000mm) causing very heterogenous hydrological conditions in space and time. This scenario considers relevant land use and land cover information and a representative hydrological characterisation of the area.

The study comprises a set of PPP application experiments (herbicide applications typical for crop rotations including alfalfa, cereals and oilseed rape) to investigate the landscape-scale impacts on *Lemna sp.* using a toxicokinetic–toxicodynamic (TKTD) model with an extensive set of existing surface water scenarios as external forcings to drive the model. The model results are used to predict the EPx, effect profile multiplier, using the 7-days moving time window approach to assess the margin of safety of each of the surface water scenarios.

Overall, the results highlight the possibilities and limitations of linking exposure and effect modelling at the landscape level in a regulatory context by expanding the existing modular open-source model xAquaticRisk.

#### **4.04.P-We327 Predicting Extinctions in Food Webs**

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Risk assessors generally rely on extrapolation methods to extend toxicity predictions from a handful of studies on standard test organisms to long-term effects for entire ecosystems. However, the question remains as to whether endpoints used in these systems translate into broader ecosystem-level scenarios. A commonly-used endpoint for ecosystems is the extinction of the species within.

Previous research on predicting species extinctions in food webs has utilised either the food web's properties or information on species dynamics (e.g., changes in biomass, early warning signals) to assess extinction risk. However, it's unknown how these properties compare to each other, and the impact on their relative performance of how far in advance extinction is being predicted. This study compares the predictive performance of biomass metrics, early warning signals and network properties within the same time series using a multi-species biomass dynamics model and a series of generated food webs subjected to simulated stress.

Extinctions were predicted from data cut-off increasingly far away from the extinction event to determine how far in advance reliable predictions could be made by different predictors, and how their relative influence changed. Our findings suggest that while biomass trends initially dominated predictions, network properties and early warning signals became more influential predictors as the time between the last point of observation and the extinction event increased.

#### 4.05 Emerging Remediation Technologies for Contaminated Environmental Matrices

##### 4.05.T-01 Evaluation of Intensified Constructed Wetlands for the Persistent, Mobile and Toxic Compounds Removal From Groundwater and Wastewater

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The occurrence of compounds such as per- and polyfluoroalkylated substances (PFAS) and other industrial compounds in surface and groundwater (GW) bodies is a matter of concern due to their persistence, mobility and toxicity (PMT). Intensified constructed wetlands (CWs) present promising solutions for addressing PMT pollutants. However, there is scarce data on using CWs, using materials like coke, to treat polluted water. This study aims to evaluate the efficiency of utilizing coke-based intensified CWs, incorporating vegetation for the removals of six PMTs and to seek to elucidate the mechanisms behind these removals.

In order to achieve it, twelve vertical columns under down-flow conditions were designed. Each column was filled with coke of different granulometric sizes (6 columns with particle size of 5-2mm and 3 columns with 2-0.5mm) or washed sand (2mm Ø). 9 of the 12 columns were planted with *Cyperus alternifolius*. All columns were fed with real GW or secondary treated wastewater (WW) at a flow of 40 and 70mm/d. The system was operated spiking at 5µg/L (benzotriazoles and some pharmaceuticals) and 10µg/L (ultra-short and short PFAS). During the operational time, every week inlet and outlet water samples from each column were collected in order to analyse physico-chemical properties, general quality parameters, target compounds removals and their transformation-by-products with a UHPLC-HRMS. After the operational time, an adsorption experiment and 16S rRNA gene sequence were produced in order to obtain a general perspective of the total removal process.

In comparing sand with coke based CWs, coke significantly improved PMTs removal, with 89% in contrast with 50% with sand. Larger coke granules increased efficiency, hitting 89% removal, compared to 48% for 0.5-2mm granules. Vegetation boosted PMTs reduction from 34% to 89%. Coke's adsorption revealed that was less than 1% and differed on the water matrix. Columns with larger coke granules increased DNA quantification (13.7ng/µL/g) improving pollutant removal. Moreover, microbial 16S rRNA preliminary results showed that Gammaproteobacteria and Micrococcaceae are the predominant classes exclusively found in the most efficient treatment.

These results suggest that an intensified coke-based constructed wetland could be an effective natural solution to eliminate PMTs from GW and WW. These results highlight the importance of understanding the pollutant removal process to be able to completely remove them.

##### 4.05.T-02 Waste biochar sorbents as sustainable alternatives for PFAS soil stabilization

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Biochars produced from excess biomass could be used as sorbents to stabilize per and polyfluorinated alkylsubstances (PFAS) in soils, to reduce their leachable, bioavailable and bioaccessible fractions. Fossil based activated carbons generally have a better effect, but the use of biochars brings environmental benefits. Biochar properties vary greatly based on pyrolysis conditions and feedstocks used, hence it was necessary to identify key properties that determine sorbent performance for PFAS. To this end, clean wood chips (CWC), waste timber (WT) and digested (DSS-1, DSS-2) and raw (DWSS) sewage sludges were pyrolyzed at 700 °C in an industrially relevant Biogreen unit and WT was, furthermore, activated with CO<sub>2</sub> at 900 °C in a Pyreka unit. We found that nonactivated wood biochar (CWC: log K<sub>F</sub> 4.44 – 5.22 (µg kg<sup>-1</sup>) (µg L<sup>-1</sup>)<sup>-n<sub>F</sub></sup>) was in fact inferior to the sludge biochars (DSS-1 and DWSS: Log KF 3.30-5.61 (µg kg<sup>-1</sup>) (µg L<sup>-1</sup>)<sup>-n<sub>F</sub></sup>), in sorbing perfluorinated carboxylic acids (PFCAs) in batch shaking tests, due to a well developed mesoporosity (pores >1.5 nm) suited for accommodating the large PFCA molecules. Activation enhanced this system of mesopores in WT biochar, in addition to the amount of condensed aromatic carbon, which is likely responsible for hydrophobic interactions between long chain (>6xCF) PFAS and biochar surfaces, thus making excellent sorbents to reduce leaching of several legacy PFAS from field contaminated soil by up to 99% in batch shaking tests. Ex situ column tests were further used to demonstrate that although the affinity of the non activated wood biochars (CWC and WT) were high for PFAS, their capacity, as measured through available mesopores, were not sufficient. The sludge biochars (DSS-1, DSS-2, and DWSS) and activated biochar (aWT), however, efficiently reduced PFOS leaching by >90% at doses of only 1%. Meanwhile, as waste biochars do not necessarily retain short chain

PFAS sufficiently, future work should be focused on biochar modifications or sequential treatments that increase the sorption of said compounds. Nevertheless, this work has demonstrated the potential for circular economy value chain creation in waste handling and soil remediation.

#### **4.05.T-03 Scale-up of a photocatalytic reactor for the degradation of pesticides at source**

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Heterogenous photocatalysis is a water treatment technology that has been widely explored in research. The scale-up of this technology, however, has been a challenge due to several reasons. First, nano powder catalysts are usually applied during photocatalysis at bench scale, therefore, it is very difficult to separate the powdered catalyst from water after the treatment is complete. Another problem to overcome is the high cost associated with the illumination of catalysts, which is usually achieved by ultraviolet irradiation (UV). Both acquisition and energy consumption of UV irradiation increase the costs of photocatalysis application. The aim of this study is to develop an economical photocatalytic treatment unit for the removal of pesticides at source from aquatic environments.

Porous beads made of recycled glass were used as an immobilization matrix for coating with graphitic carbon nitride ( $g\text{-C}_3\text{N}_4$ ) as the catalyst. The coated beads could be removed from the water and reused after each treatment cycle. The catalyst was activated using ultraviolet light with a wavelength of 365 nm (UV-A) in the format of light emitting diodes (LEDs), which is an economical alternative for UV irradiation with a long life-span. After the selection of catalyst and light source, UV-A LEDs and  $g\text{-C}_3\text{N}_4$  coated beads were tested on the photocatalytic removal of a mixture with nine pesticides (1 mg L<sup>-1</sup> each in artificial fresh water; acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam, diuron, atrazine, dimethoate and 2,4-dichlorophenoxyacetic acid). Pesticide concentration was determined by high-performance liquid chromatography coupled to photo diode array detector (HPLC-PDA).

All pesticides were completely removed within 24 hours of treatment (e.g., diuron with rate constant  $k=0.021\text{ min}^{-1}$  and 2,4-dichlorophenoxyacetic acid  $k=0.011\text{ min}^{-1}$ ), except from acetamiprid which was completely removed within 48 hours (rate constant  $k=0.0004\text{ min}^{-1}$ ). Photocatalysis based on  $g\text{-C}_3\text{N}_4$  coated beads illuminated by UV-A 365 nm LED irradiation was demonstrated to be a potential approach in the removal of pesticides from aquatic environments. Other factors also need to be taken into consideration for the scale-up of this technology, such as the system waterproofing. All materials used need to be made of stainless steel to avoid corrosion. Also, epoxy resins with high resistance to water pressure and other water components should be used to seal electrical components.

#### **4.05.T-04 Selective Degradation of Organic Micropollutants and Organic Metabolites in Source-Separated Human Urine using Sulphate-radical-based Advanced Oxidation Process**

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Human urine contains plant-essential nutrients like urea and phosphate which can be recycled back to farmland as crop fertilizer. However, food industry actors are concerned about organic micropollutants (OMPs) like pharmaceutical drugs which are also excreted in human urine, as they could potentially pose a health risk to consumers of food grown using urine-based fertilizers. This study aimed at addressing this gap by developing an Advanced Oxidation Process (AOP) that selectively oxidized >30 different OMPs in real human urine without loss on plant-essential nutrients like urea. The fate of >30 OMPs, >200 organic metabolites, and plant-essential nutrients in urine after oxidative treatment was determined.

The first set of experiments evaluated the effect of process parameters (temperature, dose of sodium persulfate, type of urine and time) on the activation of persulfate as well as the fate of COD, urea, total ammonia nitrogen, total nitrogen, and chloride in urine. In addition, targeted quantitative metabolomics were conducted to analyze the fate of >200 organic metabolites in urine by using a combination of direct injection mass spectrometry with a reverse-phase LC-MS/MS custom assay and an ABSciex 5500 QTrap mass spectrometer. The second set of experiments aimed to evaluate the fate of >30 OMPs in urine during sulfate-radical-based AOP, and analyzed using ultra-high pressure liquid chromatography tandem mass spectrometry.

The experiments resulted in an AOP where 85% of the sodium persulfate was activated, COD removal of 23%, urea recovery of 90%, total nitrogen recovery of 95% and Cl<sup>-</sup> loss of 3%. Additionally, metabolomic analysis of treated urine showed that on average, 73% of the organic metabolites were degraded by the sulphate-radical based AOP. Moreover, the average  $\Sigma$ OMP degradation in urine by the AOP was 75%. If only the OMPs listed in the new EU water directive are considered, then average  $\Sigma$ OMP degradation was 81%.

Overall, this study demonstrated the feasibility of heat-activated sodium persulfate-based AOP to selectively and preferentially oxidise OMPs and organic metabolites over plant-essential nutrients in human urine. It is evident that sulphate-radical-based AOP favors oxidation of OMPs > organic metabolites > macronutrients. The results also show that treating human urine using the developed AOP degrades 81% of the OMPs and thus meets the criteria listed in the new EU water directive on the safe reuse of wastewater in society.

#### **4.05.T-05 Mesocosms and multiparametric approaches for the environmental assessment of technologies aiming to reduce the impact of mining activities**

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Mining industry is a generator of waste in the EU and may cause considerable impacts on aquatic ecosystems. One of these impacts are the release of metals and the acid mine drainage that flows into aquatic ecosystems. Several technologies have been developed to mitigate the impact of mining but there is not a standardized method to assess whether these technologies suppose a significant improvement in water and environmental quality. To cover this gap, we have developed an easy, reliable and cost-effective mesocosms approach based on the analysis of functional and structural parameters in freshwater biofilms.

Our approach consists in artificial streams with 5L of capacity and controlled flow. Experiments are performed in a chamber with control temperature and light. Natural biofilm inoculum for experiments is collected in reference pristine rivers to growth and develop biofilm communities in sandblasted glass substratum. Experimental treatments usually consist in a control condition, raw effluents of the mine and treated effluents and exposure period last for 2 weeks. Biological responses observed when exposing communities to their respective untreated and treated mine effluent are compared with those observed in the control condition.

We are going to focus in two experiments to test the reduction of the ecological impacts achieved by treatment of mining effluents with different technologies. Exp. 1 compared effects of the raw effluent with high conductivity and concentration of metals with those of the same effluent treated with reverse osmosis. Exp. 2 compared effects of the raw effluent with high concentration of metals with those of the effluent treated by nanofiltration and electrocoagulation. Functional (photosynthetic efficiency) and structural parameters (total biomass/cm<sup>2</sup>, total Chl. *a*/cm<sup>2</sup> and community composition) of the biofilm were measured after 14 days of exposure.

The use of communities taxonomically diverse and the measurement of functional and structural parameters helps in the obtention of ecologically relevant results in the environmental assessment of these technologies because: i) this approach covers different modes of action of toxicants at different levels of biological organization; ii) key communities for the sustenance of aquatic food webs are assessed; iii) acute and chronic responses are integrated.

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#### **4.05.P Emerging Remediation Technologies for Contaminated Environmental Matrices**

##### **4.05.P-Mo341 Testing a biochar filter for removal of a pesticide cocktail and nutrients at environmentally relevant concentrations**

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Drainage systems are widely used to remove excess water from agricultural fields. In Sweden, about 47% of arable land has subsurface drainage systems installed. Throughout the growing season, these systems inadvertently transport pesticides and nutrients to the aquatic environments downstream. Implementing cheap and effective filters directly connected to the drainage systems has significant potential to reduce such transport.

We aim to develop a cost-effective filter, designed from readily available organic materials, to reduce pesticide transport into surface water, contributing to sustainable agricultural practices. Consequently, the initial phase of our research involved testing various materials (such as biochar, sand, wood chips, straw, Leca, etc.) in laboratory columns using different surface waters spiked with a diverse pesticide mix (up to 160 plant protection substances) at environmentally relevant concentrations (0,1-1 µg/L).

In our preliminary investigations, one particular biochar, exhibiting a BET surface area of 334.6 (m<sup>2</sup>/g), outperformed all other materials tested. It demonstrated a remarkable concentration reduction of >95% for all 108 measured pesticides from inlet to outlet. The capacity test revealed that a 10 g quantity of this biochar in the filter could maintain a concentration reduction of >95% for a pesticide cocktail mixture in up to 8 L of surface water (equivalent to 223 pore volumes) with low organic content (DOC 4.9 mg/L). Additionally, it demonstrated effectiveness in high-organic-content surface water (DOC 27 mg/L), sustaining a concentration reduction of >95% in up to 4 L (equivalent to 441 pore volumes).

Subsequent stages of this study will involve a more comprehensive capacity test and a field campaign where the filter will be deployed in drainage pipes. We will closely monitor its performance in removing pesticides and nutrients. Our ultimate goal is to provide a practical solution for reducing pesticide transport, thereby enhancing water quality and the ecological well-being of aquatic environments within agricultural landscapes.



#### **4.05.P-Mo342 Synthesis of Granules from Polyaluminum Chloride (PAC) Sludges for Environmental Application in As(V) Removal: Influence of Calcination Temperature on As(V) Adsorption Ability**

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Arsenic (As) contamination in soil/groundwater is one of the challenging global issues that should be urgently addressed due to its severe environmental risk. Specifically, the need to properly remove arsenate (As(V)) from aqueous system is strongly emphasized in developing countries that adopt groundwater as drinking water. In terms of the environmental sustainability, it is highly reasonable to utilize waste-derived materials as raw feedstock for the development of functional adsorbent. In this study, we aimed to fabricate granular As(V) adsorbent by calcination of polyaluminum chloride (PAC) sludge, and optimize the calcination temperature by focusing on enhancement of As(V) adsorption in batch- and column modes. The physicochemical characteristics of calcined sludges were analyzed by XRD, XRF, BET/BJH, TGA, and Zeta-potential instruments. Porous framework creation in the sludge matrix were achieved through the calcination process up to 500°C, but the adsorbents fabricated at temperature above 500°C exhibited less adsorption of As(V) due to crystalline phase transformation. By the comparison of As(V) adsorption, the sludge calcined at 500°C (PA500) was chosen as an optimal adsorbent. The adsorption of As(V) by PA500 was terminated within 6 h, and its maximum adsorption capacity was found to be 15.9 mg/g in the Langmuir isotherm model fitting results. The adsorption of As(V) exhibited the low pH dependence and was highly impacted by the existence of P(V) in form of PO<sub>4</sub><sup>3-</sup>. The column test results represented that a column filled with 7 g PA500 successfully controlled As(V) to below 10 µg/L during 200-h operation at 50 µg/L As(V) and 1 mL/min flow rate. The stability of calcination process was well demonstrated with less leaching of trace metals from the sludge at 500°C calcination and exposure to >pH 3 solution. In conclusion, calcination of PAC sludge at optimal temperature can be a promising method to fabricate As(V) adsorbent.

#### **4.05.P-Mo343 Removal of diclofenac by filtration: experimental results and modelling**

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The occurrence of organic micropollutants in surface waters is of serious concern because of their ubiquity and adverse effects on humans and environment. Wastewater treatment plants are the main disposal of these pollutants into the environment due to their inefficient removal in the course of water purification. The European Union established a watch list of substances for their potential inclusion in a list of priority substances in the field of water policy, in which diclofenac (DC) was included (Decision 2015/495/EC). Recently, the new EU Directive proposal for urban wastewater treatment (2022/0345) set a list of 16 substances, including DC stayed, that classified them into three categories based on ease of their removal. The analysis of removal of DC by using bed filtration with two different adsorbents was analyzed by a model that considers convection and adsorption/desorption, which was extended by accounting for degradation during filtration. The sorbents used were a granular activated carbon (GAC) and an enzyme-based complex. The validity of the model was demonstrated. In the particular case of the enzyme-based complex, the removal of DC is initially due mostly to adsorption and hardly degradation. The model yields in parallel to the experimental results an increase of the concentration of emerging DC with time for a range of times, but the model predicts attainment of a steady state which is reached earlier in the case of a larger degradation constant and in the case of a longer filter. The analysis of the filtration data with the model enabled to determine parameters for generating predictions for other sets of operational conditions. This enables to determine the capacity of the filters for economic estimates.

#### **4.05.P-Mo344 Assessment of Novel Constructed Wetland Configurations for Preventing Groundwater Pollution from Contaminants of Emerging Concern. A Bench-Scale Study**

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Nature-based solutions, such as constructed wetlands, have shown to be effective and sustainable technologies to remove contaminants of emerging concern (CEC) from wastewater, becoming feasible measures to prevent groundwater pollution. In consequence, interest has grown in finding new configurations and materials to increase their efficiency. Within the context of UPWATER project, this study assessed the effectiveness of novel constructed wetland designs on CEC mitigation during groundwater recharge with secondary treated wastewater. Configurations tested included: floating root mats (FRM) with: *Cyperus alternifolius*, *Cyperus alternifolius* with biochar and control pools, and horizontal flow constructed wetlands (HFCW) where *Phragmites australis* performance was tested in three filling materials: biochar, coke, and sand. Experiments were fed with secondary treated wastewater spiked at an environmentally relevant concentration (5 µg/L) with the 10 most relevant pollutants in groundwater bodies (benzotriazole, bisphenol A, carbamazepine, diclofenac, gemfibrozil, iopamidol, lamotrigine, methyl benzotriazole, sucralose, and valsartan).

Among FRM configurations, best removals were achieved in the *Cyperus* system with biochar, ranging from 41% for iopamidol to 99% for gemfibrozil (82% on average) for Hydraulic Loading Rate (HLR) 12 mm/day and ranging from 23% for sucralose to 99% for gemfibrozil (72% on average) for HLR 24 mm/day. Configurations with only *Cyperus* obtained an average of 62% and 58% with HLR 12 and 24 mm/day respectively, whilst control setups gave an average of 50% and 34% with HLR 12 and 24 mm/day. Among HFCW configurations, biochar stood out as the most effective material, achieving an average of 98% and 97% removal efficiency for HLR 50 and 100 mm/day, with significantly higher removals for 7 out of 10 pollutants compared to other treatments. Coke resulted in removal average of 59 and 45% and sand of 60 and 52% for HLR 50

and 100 mm/day respectively. The addition of ZVI did not result in better removal efficiencies, on average. These results underscore the significance of the filling material choice in the design of constructed wetlands for CEC removal from wastewater, with biochar offering the most effective solution, they prove the effectiveness of plant presence and the importance of HLR choice. Thus, these experiments highlight the viability of nature-based solutions as a promising measure for preventing groundwater pollution.

#### **4.05.P-Mo345 Nature-Based Solutions to Reduce Antibiotics, Antimicrobial Resistance Genes and Risk Assessment Approach**

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Climate change and high water demand generated by human population growth and the continuous expansion of industry and agricultural activities reduce the quality and quantity of available freshwater years. In recent years, antibiotics (AB) have become the primary concern due to the increased concentration in aquatic environments. ABs are able to reach the aquatic stream via wastewater treatment plant (WWTP) discharge and livestock manure application. Furthermore, antimicrobial-resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) are also present in wastewater and may pose a rising risk to aquatic ecosystems and human health concerns due to the reuse of this water.

In this study, 12 conventional wastewater quality parameters and 22 ABs for 9 different categories (beta-lactam, trimethoprim, fluoroquinolones, ionophore, lincosamides, macrolides, rifamycin, sulfonamides and tetracyclines) were monitored in the seasonal performance (summer and winter) on 2 different CW configurations (Surface Flow (SFCW) and Horizontal Flow (HFCWs)) in terms of improvement of general water quality parameters and reduction of ABs, ARGs and risk assessments.

NBSs were able to reduce the release of nutrients (N and P) into the aquatic environment better than the conventional TWWTP reference. 12 out of the 22 ABs were detected with a concentration ranging from 5 to 1,218 ng·L<sup>-1</sup>. Azithromycin (AZI) was the most abundant AB with a concentration level above 400 ng·L<sup>-1</sup>. A moderate to high attenuation capacity for ABs was performed by NBSs (74% to 88% for both seasons on average) than conventional T-WWTP (34% for both seasons on average). Moreover, preliminary results showed a reduction of ARG in NBSs (intl1 and sul1) by 99% and less than 90% for TWWTP. Regarding risk assessments, ecotoxicological and potential bacterial selection risks (PMRS) were estimated at influent and effluent points for each treatment. Results indicated a high accumulative risk ranging from 4.7 to 70.1 at influent points. NBSs were able to minimize the risks (61 to 87% for both seasons on average) more than conventional TWWTP technology (<20% for both seasons on average). Nevertheless, only SFCW was able to reduce the cumulative risk for PMRS below 1 during the warm ( $\Sigma RQs = 0.8$ ) and the cold periods ( $\Sigma RQs = 0.8$ ).

In conclusion, this study shows that NBS can be employed as a tertiary treatment technology to improve water quality parameters and to reduce ABs, ARGs and risks in aquatic ecosystems.

#### **4.05.P-Mo346 Cost-Benefit Analysis of Innovative Microplastic Filtration System**

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Approximately 35% of microplastics released to oceans globally originate from washing synthetic textiles. Therefore, there is a growing need for effective removal technologies to facilitate the reuse of greywater and effectively preventing the discharge of microplastics. In this study the comprehensive cost-benefit analysis (CBA) of innovative filtration system with membrane pores as small as 0.1 microns designed specifically for the removal of microplastics from wastewater sources is performed. This innovative microplastics filtration system maintains low pressure while allowing high water permeability and the flow and finally a sustainable wastewater treatment system. This innovation allows for the comprehensive removal of fibers or particles ranging from 5 mm to 0.10 microns in a single automated process, conserving 70% more energy. The CBA analysis included different factors such as the initial capital investment for filter installation, operational and maintenance costs, as well as the potential benefits derived from reduced environmental impact and improved water quality. Environmental benefits are assessed through the estimation of microplastic removal efficiency and subsequent positive impacts on aquatic ecosystems. This cost-benefit analysis contributes valuable insights into the economic viability of microplastic removal filters, providing a foundation for informed decision-making by policymakers, environmental agencies, and stakeholders involved in water management. The results aim to guide the development of sustainable strategies for mitigating the impact of microplastic pollution on water ecosystems, human health, and the global environment.

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#### **4.05.P-Mo347 At-source hospital wastewater treatment to eliminate harmful pharmaceuticals: a novel immobilised approach using UV-LED activated photocatalytic nanomaterials**

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After excretion by hospital patients, drug concentrations in hospital wastewater are very low (mostly in the ng L<sup>-1</sup> concentration range). Nevertheless, drugs released from hospitals pose an increasing risk to the aquatic environment and to human health, as ecotoxic hospital drugs and their metabolites accumulate in food chains. In addition, accumulation of hospital-derived antibiotics in the environment contributes to the growing problem of antimicrobial resistance. Current conventional wastewater treatment still fails to effectively eliminate most persistent hospital drugs and research into novel, sustainable and economical treatment technologies is urgently needed to eliminate the complex mixture of toxic drug residues in hospital wastewater.

Immobilised photocatalysis, using economical UV-A LEDs and light-activated metallic nanomaterials may provide a sustainable, reusable at-source hospital wastewater treatment solution for the oxidation and elimination of toxic hospital-derived drugs. At bench-scale, high-temperature calcination of photocatalysts onto spherical supports, such as glass beads, is mostly used to incorporate photocatalysts onto solid (porous) structures. However, the use of spherical support materials in fast-flowing hospital wastewater can cause unwanted hydrodynamic effects. In this study, planar borosilicate support materials were functionalised with an effective photocatalytic nanomaterial thin-film and tested in a novel UV-light fixed-bed reactor.

A wide band gap 3.37eV UV-light active photocatalytic metallic nanomaterial, zinc oxide (ZnO) and, by way of a negative control, narrow band gap 1.24 eV UV-light inactive, copper (II) oxide (CuO), were covalently functionalized and thin-film coated onto a borosilicate glass support by means of silanization using 3-aminopropyltriethoxysilane (APTES). Widely applicable borosilicate is a promising support matrix for photocatalytic applications in novel continuous flow treatment settings, such as for the effective oxidation and elimination of hospital drugs from fast-flowing wastewater. Furthermore, economical 365nm UV-A LEDs provide a cost-effective low energy alternative to conventional xenon light bulbs.

This novel immobilised photocatalytic treatment design has demonstrated tremendous effectiveness to oxidise and eliminate various toxic hospital drugs in fast-flowing simulated wastewater, by means of UV-light activated synthesis of reactive oxygen species (ROS) on the catalyst surface.

#### **4.05.P-Mo348 Leaching experiments in columns systems to study the potential use of organic wastes as organic amendments to reduce groundwater pollution.**

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The use of pesticides in agriculture is necessary for large-scale food production and to keep populations supplied. However, its intensive use is a source of pollution in the different environmental compartments. The addition of organic matter to soils could help to control the migration of soils into groundwater, as organic matter has been shown to be able to adsorb them. In this work, the potential use of several low-cost organic wastes (chicken manure - CM, rice husk - RH, composted urban solid waste - CRSU and residues from cotton gins - CG) was studied as soil amendments using leaching columns at laboratory scale. Hand packed columns were constructed from soil and soil amended with 10% of the cited organic wastes. Pesticides of different physico-chemical characteristics (Atrazine-AT, chlorfenvinphos-CF, chlorpyrifos-CP, simazine-SM and trifluralin-TF) were applied at the beginning of the experiment and columns were subjected to a simulated rain till pesticide concentration in the leachate remained constant. Pesticides were extracted and analyzed by Stir Bar Sorptive Extraction and analyzed by Gas Chromatography coupled with Mass Spectrometry. Relative and cumulative breakthrough curves (BTC) were constructed from pesticide detected each day in aqueous leachate. In general, the day of elution was delayed by amending the soil, with a few exceptions where it was brought forward or had no effect. Sorption of the organophosphate CF, improved with all studied organic residues, both in BTC and cumulative curves. The cumulative curves showed that the addition of the proposed organic residues improves the retention of the pesticide CF by up to 2.5 times. CG doubles the adsorption capacity of the soil and CM is good to retain the herbicide SM. The addition of organic residues does not appear to have any effect on soil holding capacity for the low-water solubility pesticides CP and TF. Results showed that specific organic waste incorporation in soil could reduce the leaching of pesticides.

#### **4.05.P-Mo349 Contribution of earthworms and plants to remediation of contaminated shooting range soil**

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Shooting sites are a major concern due to high levels of pollution. As ecosystem engineers, earthworms can help maintain soil structure, improve soil quality, and alter the availability of heavy metals. Therefore, it can be used as in phytoremediation processes. Plants and earthworms were exposed to different levels of soil contamination. Our aim was to evaluate the role of earthworms and plants in soil metal availability and their influence on plant metal uptake at different heavy metal concentrations in soil. The results show that earthworms and plants changed the level of metals in the contaminated soil after exposure. Reproduction was more affected by contamination than worm survival or weight change. Metal accumulation in

plants depended on the metal element under consideration and the presence of earthworms. These findings revealed that earthworm activity can alter the availability of heavy metals to plants in contaminated soil.

#### **4.05.P-Mo350 Evaluation of the Improvement in Urban Runoff Water Quality After Passing Through Sustainable Urban Drainage Systems (SUDS)**

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When rain falls on a natural landscape, it infiltrates into the soil, evaporates, is absorbed by plants and part of it reaches streams and rivers. These stages of the water cycle can be prevented when land is altered by urban development, due to the fact that there is less permeable ground available for infiltration. When rain falls on impermeable surfaces, more surface water runoff is generated, which can cause floods, water pollution and erosion problems. Climate change predictions show it is likely that heavy rainfall and flooding will become more frequent, so, it is important an effective management of surface water runoff. In this context, Sustainable Urban Drainage Systems (SUDS) have been designed to maintain and protect the natural water cycle, to support the management of flood risk and to manage the quality of the runoff to prevent pollution. Among these systems, permeable pavements allow rainwater to infiltrate through the soil and stormwater tanks can store the runoff water for its later use with another purpose, such as for irrigation or cleaning activities.

The study presented here is located in Legazpi, a town of the north of Spain which participated in a project called LIFE Good Local Adapt. The goal of this project is to develop sustainable solutions to adapt the city's neighbourhoods to extreme weather events and its consequent floods. With this aim, permeable pavements were installed in one of the parkings of the town of Legazpi, which were connected to a stormwater tank. The objective of this study was to evaluate the influence of SUDS on the quality of urban runoff and for this purpose, several physicochemical parameters (pH, turbidity, conductivity, dissolved oxygen) and the content of anions, metals, metalloids and Polycyclic Aromatic Hydrocarbons (PAHs) were determined in urban waters. The water before entering the permeable pavements, after passing through the permeable pavements and the water stored in the stormwater tank was taken at the beginning of different rainy events. Therefore, urban waters were compared before and after passing through the SUDS. Results show a decrease in the turbidity of the waters after passing through the permeable pavements and a reduction of the concentration of some metals, such as iron and copper. Despite this improvement, the water storing in the tank results in a possible accumulation of some elements, such as chlorides or lithium.

#### **4.05.P-Mo351 Evaluation of the Influence of Hydrodynamic Separators on the Quality of Urban Runoff**

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Anthropic sources and soil or rock degradation form a very important matrix: the sediment that can be found in urban areas. These urban sediments accumulate different types of pollutants, until they are pulled by urban runoff. Urbanization implies an increase in the waterproofing of the land surface, which increases the volume of urban runoff and the speed at which this moves through the urban surface, favoring the drag of urban sediments, and thus, its contaminants. Sustainable Urban Drainage Systems (SUDS) have been implemented with the aim of preventing floods and protecting and improving the quality of urban runoff, mitigating the impact that pollutants of the urban sediment produce on the discharge point into the natural channels. Hydrocyclones or hydrodynamic separators can be classified as treatment systems that are designed for the physical removal of the sediment of urban runoff by gravity, moving the water in a centrifugal manner from the inlet to the outlet.

The main objective of this study was to evaluate the influence of hydrocyclones on the quality of urban runoff in different sites and rainy events. Water samples were collected at the inlet and outlet of five hydrocyclones located in San Sebastian, a city located on the Cantabrian coast, in northern Spain. Three hydrocyclones (H1, H2, H3) are located in an industrial polygon of the city, one in a residential area (H4) and another in a residential and touristic area next to the port (H5). Comparing the inlet and outlet water of the hydrocyclones, it was not observed a general trend of decreasing contaminant content. Turbidity was generally related to the content of metals and metalloids but not to anions and it generally decreased in H1, H2 and H4, but not in H3 and H5. Comparing the sites, the waters of H1 and H2 had higher content of Zn and H4 contained more sulphates and nitrates. Samples of May contained more contaminants than in November and this difference could be related to the precipitation of the previous days: the rainfall accumulated in 7 days before the samplings was 0.3 mm and 57.6 mm, respectively. So far, it has been observed that the amount of rainfall in the previous days influenced the contaminant content and that the inlet and output results varied according to the hydrocyclone, the sampling day and the contaminant. Further samplings are required in order to understand better the functioning of these systems.

#### **4.05.P-Mo352 Assessment of the Influence of Permeable Pavements on Urban Runoff Quality: a Case Study in San Sebastian (Spain)**

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In recent decades, the urbanization of cities has caused an increase in surface waterproofing, which has modified the natural water cycle, preventing, between other things, the infiltration of surface waters to the ground. This fact increases significantly the volume of urban runoff and the speed at which this moves through the urban surface, favoring the drag of urban sediments. Sustainable Urban Drainage Systems (SUDS) have been implemented with the aim of preventing floods and protecting and improving the quality of urban runoff. This way, they can mitigate the impact that pollutants present on the urban sediment can produce on the discharge point into the natural channels. Permeable Pavements are a type of SUDS that favor the infiltration of urban runoff and PICP (Permeable Interlocking Concrete Pavement) and PA (Pervious Asphalt) are among the most employed structures. In PICP urban runoff is infiltrated through the joints between blocks and in PA through a porous material. Since runoff water is infiltrated through the pavement pores, a filtration process is generated, which can improve the quality of the infiltrated water.

The objective of this study was to evaluate the influence of permeable pavements on the quality of urban runoff. The study site is a parking located in a residential district of San Sebastian (north of Spain) and includes two types of permeable pavements: PICP and PA. In addition, the area offers the opportunity to compare the runoff before and after passing through the permeable pavements, since it includes a sump with three channels, on which water that comes directly from the surface and water that has passed through each of the permeable pavements is differentiated. For the evaluation of the influence of permeable pavements on the quality of urban runoff, several physicochemical parameters were measured in the water collected from each of the three channels mentioned: turbidity with a turbidimeter and pH, conductivity and dissolved oxygen using a portable series meter. Then, anions were determined by Ion Chromatography (IC) and metals and metalloids by an Inductively Coupled Mass Spectrometer (ICP-MS). Results showed a decrease in the content of some metals (such as Fe, Ba and Mn) after passing through the SUDS, and a clear difference was detected in the turbidity of the surface waters (39 FNU) and the waters that passed through the two types of permeable pavements (1.6 FNU).

#### **4.05.P-Mo353 Bacteria Responsible for Disruption of Bentonite Barrier in Hazardous Waste Landfill**

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Although the number of landfills will decrease in the some waste, especially toxic waste, including incinerator fly ash, will still be landfilled. The structural elements of landfills often include sealing geosynthetic clay (bentonite) layers to protect the environment against toxic leachates. The extreme chemical properties of hazardous landfill leachate together with the high activity of microorganisms affect the bentonite mineralogy and isolation properties. As an example, montmorillonite with high swelling capacity can be replaced by non-swelling kaolinite and illite. This can lead to the leakage of toxic substances into subsoil and groundwater. Our study aims to determine the bacterial populations proliferating in the bentonite saturated with hazardous waste landfill leachate and affecting bentonite isolation properties.

Freshly collected landfill leachate from the hazardous waste landfill was transported to the laboratory and mixed with bentonite. Mixtures were airtight and incubated at 50 °C for three months in a self-developed anaerobic atmosphere. Vessels containing bentonite mixed with tap water and sterilized (Gamma irradiated) landfill leachate were added to the same heated box and served as controls. At the end of the incubation, samples were collected for DNA extraction and tests of chemical, geo-mechanical and hydro-dynamical properties of bentonite. Extracted DNA samples were used for qPCR and DNA sequencing analyses to compare the abundance and composition of bacterial communities in bentonite mixtures.

Most bentonite samples had higher bacterial abundance after the incubation than at the beginning. Bentonite mixed with the landfill leachate contained high amounts of acetogenic, iron-reducing, and sulphate-reducing bacteria. Some bacteria were present in the landfill leachate, but their abundance decreased during incubation, e.g., halophilic *Halocella* and *Marinebacterium*. On the contrary, others increased during incubation, such as the halophilic *Marinococcaceae*, *Sinibacillus* and *Oceanobacillus*. The most abundant taxon within the community were bacteria from the *Limnochordaceae*, which ferment organic substrate. Particularly sulphate, sulphite and iron-reducing bacteria from the landfill leachate contributed to the disruption of the bentonite layer.

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#### **4.05.P-Mo354 Enhancing hospital wastewater treatment: evaluating the combined efficacy of a Moving Bed Biofilm Reactor (MBBR) and nanofiltration to mitigate effluent risks.**

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Hospital wastewater contain potentially harmful molecules, which pose environmental and health hazards. Traditional wastewater treatment methods are not effective in the elimination of some micropollutants such as pharmaceuticals, personal care products, and other toxic substances. This study investigates the use of a Moving Bed Biofilm Reactor (MBBR) combined with nanofiltration to effectively reduce contaminant release and effluents ecotoxic potential. A non-targeted LC-MS-MS analysis was carried out to monitor the effluent contaminant composition and treatment process efficiency. Thus, 1235 molecules were detected in the influent and 349 after treatment. Among the molecules identified based on the highest reliability score, 71 and 34 were identified in the influent and treated effluent respectively. On the remaining molecules, 50% exhibit a relative reduction rate exceeding 75%, indicating the effectiveness of this hybrid system. Two standardized assays were carried out to determine efficiency on the effluent toxicity: an acute test with *Daphnia magna* on raw effluent and a (geno)toxicity test with *Xenopus laevis* using dilutions representative of WWTP environmental releases. The acute toxicity test showed treatment efficiency, leading to a reduction of *D. magna* mortality induced by influent exposure. In *X. laevis*, no genotoxicity of the matrix was demonstrated whatever the condition. However, a significant reduction of erythrocytes mitosis was observed even at the most diluted concentration of effluent (1/300). Significant growth inhibition was observed for the untreated effluent diluted at 1/10, while not after exposure to diluted effluent (1/10). No impact on growth was observed at 1/300 dilution, either before or after treatment. Analysis of gut microbial diversity using 16s RNA sequencing showed significant changes in microbial population, suggesting potential physiological impacts on organisms. Overall, the hybrid MBBR/nanofiltration system effectively lowers micropollutants in hospital effluents and reduces ecotoxicity. Yet, ecotoxicological assays show residual toxicity to *Daphnia m.* and sublethal effects on *Xenopus l.*, highlighting the necessity of integrating chemical and ecotoxicological evaluations for a comprehensive assessment of the environmental impact of treated hospital effluents.

#### **4.05.P-Mo355 Reduction of Eco-toxicity of Biodegradation of the Antidepressant Citalopram by Co-metabolically Stimulated Polishing Moving Bed Biofilm Reactors**

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Citalopram is one of the most utilized antidepressants around the world. Citalopram and its transformation products (TPs) are frequently detected as in natural water due to the inefficient wastewater treatments for them. Polishing moving bed biofilm reactors (MBBRs) were able to remove citalopram efficiently according to previously studies, however the TPs and their ecotoxicity were not comprehensively evaluated.

This study investigated the biotransformation efficiency and pathways of citalopram by polishing MBBRs in a laboratory batch experiment. Through target and non-target analysis by HPLC-HRMS, the transformation rate and TPs of citalopram were comprehensively analyzed. The eco-toxicity of TPs was predicted using ECOSAR.

Of the 19 TPs, 7 were de-novo identified. These included quaternary amines, alkenes and conjugate TPs. Notably, dosing simple carbon source, i.e., acetate as a co-metabolically stimulation did not only increase the reaction rates of citalopram, but largely varied the transformation pathways. The nitrile hydrolysis (up to 43%) was the major pathway under carbon-limited condition, whereas amide hydrolysis and *N*-oxidation became the main pathways under carbon-rich condition. Moreover, carbon stimulation indicated towards formation of less toxic metabolites, i.e., carboxylic acid, *N*-oxide and ester TPs of citalopram, while the carbon-limited condition resulted in the more toxic carboxamide, *N*-desmethyl and alkene TPs. Therefore, this study proved that a co-metabolic stimulation by dosing simple carbon source to polishing biofilm could lead to a more efficient reduction the eco-toxicity of citalopram TPs than the constant carbon starvation condition.

#### **4.05.P-Mo356 Sustainable support liquid membranes based on deep eutectic solvents for the removal of contaminants of emerging concern from WWTPs effluents**

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Contaminants of emerging concern (CECs) such as UV-filters, pharmaceuticals and pesticide residues in both wastewater treatment plants (WWTP) effluents and surface waters are associated with the increasing urbanization and limited removal efficiency of CECs by WWTP and represent a great threat to the environment and human health. Despite the availability of some treatment technologies, e.g., membranes bioreactors and ozonation to remove CECs the majorities of these treatments are expensive and their application limited.

Deep eutectic solvents are biodegradable solvents that have been used to efficiently extraction of CECs from the sample medium. The immobilization of organic solvents in membranes permits the separation of large amounts of contaminants with low consumption amount of solvent. However, most of this solvent are toxic an present low stability which constrained its industrial application. Therefore, in this work, we will present the main objective of the CLEANH2O project, which aims to provide a sustainable and cost-effective technology using a supported liquid membrane based on deep eutectic solvents for the removal of relevant CECs from WWTP.

#### **4.05.P-Mo358 Unraveling Mercury Stable Isotope Ratio Fractionation from Mine Waste to Fish**

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Elevated levels of mercury (Hg) are frequently found at locations adjacent to and downstream of legacy mercury mines. Persistent mercury impact from legacy mines continue to present risk to the environment. Understanding of the source pathways of Hg methylation (MeHg) is critical in evaluating and minimizing Hg impact to ecosystems. Our study area was the

Stibnite Mining District located near the headwaters of the East Fork South Fork Salmon River in central Idaho, USA. Cinnabar Mine drains into Cinnabar Creek, a steep and narrow mountainous drainage system ranging in elevation from mountain peaks at 8900 ft. down to 6200 ft. at the Cinnabar Creek confluence with Sugar Creek. The study area is remote with minimal contribution from other anthropogenic sources of Hg allowing for a detailed Hg source pathway assessment. Samples were collected from three sampling sites downstream of the Cinnabar Mine area and two baseline sampling sites located above the Cinnabar Creek and Sugar Creek confluence on Sugar Creek. We measured Hg concentration and isotopic composition for biological (bull trout, spiders and macroinvertebrates), calcine and sediment samples. Scanning electron microscope (SEM) imaging of seston material identified absorbed sub-micron and micron sized cinnabar and calcine particles suggesting that other samples collected from the impacted streams may also contain absorbed inorganic Hg (IHg). Fish, spider and adult macroinvertebrates contained predominantly MeHg, whereas larval macroinvertebrates collected from the impacted streams containing mostly absorbed IHg. Initial interpretation of the data identified the need for determining  $\delta^{202}\text{Hg}_{(\text{MeHg})}$  in addition to  $\delta^{202}\text{Hg}_{(\text{T})}$ . The  $\delta^{202}\text{Hg}_{(\text{MeHg})}$  was calculated by applying a mass balance formula using MeHg and  $\text{Hg}_{(\text{T})}$  concentrations as well as  $\delta^{202}\text{Hg}_{(\text{T})}$ . The  $\delta^{202}\text{Hg}_{(\text{MeHg})}$  for samples collected at the three sample sites downstream of the mine area resulted in very negative  $\delta^{202}\text{Hg}$ . A possible source of the very negative  $\delta^{202}\text{Hg}$  determined for the MeHg is gaseous Hg flux originating from large calcine waste piles. In-lab gaseous Hg flux experiments were conducted on waste calcines collected from the Cinnabar Mine area and resulted in similar negative  $\delta^{202}\text{Hg}$  similar to that determined for the MeHg in the samples. These results suggest that in this region a primary source pathway of Hg to the food web is likely gaseous Hg flux evolving from waste calcine piles.

#### **4.05.P-Mo359 Effects of pH and Competing Ions on the Sorption of Oxyanion-Forming Contaminants onto Lake Sediments**

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Oxyanions such as chromate, arsenate, vanadate, selenate, molybdate, and antimonate are often present both naturally in the environment and as a result of anthropogenic activities. Mining activities, the burning of fossil fuel, and municipal waste are contributing towards the increasing release of these oxyanions.

Despite the numerous studies in the sorption behaviour of these oxyanions, they are usually studied in a single element system with pure mineral as the sorbate. However, studies on sorption of oxyanion mixture onto natural samples are limited. This study simulates a pollution event involving these oxyanions in freshwater lakes through laboratory experiments.

We investigate the sorption of chromate, arsenate, vanadate, selenate, molybdate, and antimonate onto lake sediments using batch sorption method. A mixture of contaminant spikes containing those mentioned above were added to sediment and water sample taken from Loch Leven, Kinghorn Loch, and Loch Fitty in Scotland (UK) then shaken for about 48 hours at ambient temperature. The three lakes have different contamination history with varying mineralogical composition.

Two variables are included for the study: pH and competing ions. All contaminants are more mobile in alkaline conditions ( $\text{pH}>7$ ) and less mobile in acidic conditions ( $\text{pH}<7$ ). In the presence of phosphate ( $\text{pH}\approx 8$ ), both arsenate and vanadate are more mobile with the increase in phosphate concentration, while there is a slight increase in the sorption of antimonate. For selenate and molybdate, very low sorption is observed at pH 8, with no observed change despite the increasing phosphate concentration. No change is observed in chromate sorption with the addition of phosphate, but there is a trend of increased mobility with the addition of carbonate. For other oxyanions, the addition of carbonate does not seem to affect their sorption. Sulfate addition has little to no effect to the sorption of oxyanions.

In conclusion, the sorption of oxyanions in the sediment-water interface is affected by the pH and available competing ions in the environment. The presence of competing ions has different effect towards the sorption of oxyanion-forming contaminants. However, the behaviour of these contaminants are quite similar despite the different characteristics of the lake sediments. This study provides more insight into a real-life scenario where pollution events usually contain mixtures of contaminants rather than a single element.

#### **4.05.P-Mo360 Assessing the relevance of bauxite residue amendments with gypsum and organic wastes for phytomanagement of Bauxite Residue from Hydro-Alunorte, Brazil**

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Bauxite residue is a highly alkaline, saline, and sodic tailing of alumina refineries with little re-use potential and is mainly dry-stacked in land disposal areas. Erosion caused by wind and rain imposes the risk of hydrosphere and lithosphere contamination. In situ revegetation of the ameliorated residue is the most effective strategy for mitigating the environmental risks. The study presented aimed to assess revegetation solutions for bauxite residues from the Hydro-Alunorte refinery in Brazil, based on technosol construction using local waste materials. Column experiments were used to assess the success of soil amendments for limiting the concentrations of alkaline, saline and metal(loid) compounds in soil water. The effect of the soil amendment solution on revegetation success was assessed by focusing on the root development. Image analysis techniques were performed on micro-computed tomography ( $\mu\text{CT}$ ) scans from 5 samples that were amended by different compositions of

gypsum and local organic waste. After manual segmentation of roots from the mixtures, they were quantitatively analysed for number and depth of roots, root volume density, root area ratio, and porosity of the soil. After two weeks of growth and out of 30 seeds, up to 21 roots with average depth of 10 mm were observed across all samples; and after four weeks up to 50 roots with average depth of 12mm. Porosity values across all samples averaged into 64% before plantation, 54% after two weeks, and 37% after 4 weeks of growth. Samples with 10% gypsum and no organic amendment showed most dense root development, with clearly different root densities compared with samples with either acai or food waste. Samples with 5% gypsum had very poor to no growth. Root descriptors were correlated with certain chemical parameters such as dissolved organic carbon, electrical conductivity, alkalinity, and calcium concentration in the leachates. It was concluded that image analysis based on  $\mu$ CT is an effective method to analyse the roots in bauxite residue and that root growth is closely related to chemistry of the ameliorated residue. This work also showed that reuse of local waste fractions is a promising strategy for constructing technosols supporting vegetation for phytomanagement of bauxite residues.

#### **4.05.P-Mo361 How do Physicochemical Factors and Suspended Particulate Matter Impact the Metal Content of River Water in a Tropical Environment (New Caledonia)?**

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In New Caledonia, 61% of the sources of drinking water arise from surface water whilst 24% originate from ultramafic massifs. In these massifs, natural weathering and anthropogenic activities, (mining, fires and invasive species), enhance soil erosion and promote the production of suspended particulate matter (SPM) rich in metals (Ni, Co, Cr) into local rivers. This study examines the river water quality downstream of ultramafic massifs, with a specific focus on the role played by SPM in their water quality. Fine fractions (<100  $\mu$ m) of soils from mines and river sediments were studied because they are considered representative of SPM. Their mineralogy was characterized by X-Rays Diffraction. The samples were mineralized using acid digestion assisted with microwaves to determine their metal content. Sequential extractions were then performed on these solid samples using an adapted BCR protocol to investigate the potential transfer of metals from SPM to the dissolved phase of water during changes of physicochemical conditions (T, pH, Eh...). Mineralized samples and extracts were analyzed by ICP-AES (Cr, Fe) and ICP-MS (Ni, Mn, Co, As). Soils and sediments are mainly composed by serpentines, quartz and Fe or Mn oxides/hydroxides. They contain significant concentrations of Fe, Mn, Ni, Co and Cr (few  $\text{g}\cdot\text{kg}^{-1}$ ), exceeding the available Ni, Co, Cr quality guidelines of Europe and North America. Sequential extractions underline first that Mn and Co can be transferred from SPM to the dissolved phase of water under reductive conditions (around 37% in sediments, 60% in soils). This process is suggested to be associated to the dissolution of Fe and Mn oxides/hydroxides. Secondly, Fe, As, Cr, Ni, Mn and Co are demonstrated to be strongly retained in the solid phases, most likely in Si-rich mineral phases such as serpentine and quartz. However, whilst metals are released to a limited extent from solid samples to the water (e.g. an average of 12% of the total Ni content in the first 3 steps of sequential extractions), due to the high metal content of the bulk sediment (an average Ni concentration of  $7.1 \text{ g}\cdot\text{kg}^{-1}$ ), solid/liquid metal transfers cannot be ignored. Changes in physicochemical conditions (pH, Eh) can lead to Ni and Cr levels in water approaching the WHO's guidelines, which may pose a health issue. This study emphasizes the fundamental importance of SPM in assessing water quality and will assist in establishing appropriate water guidelines in New Caledonia.

#### **4.06.A Hazards, Risks, and Management of Soil Ecosystems for Sustainable and Environmental Conservation**

##### **4.06.A.T-01 Influence of soil properties in lower tier tests. Is an assessment factor based uniquely on Organic Carbon sufficiently protective?**

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Environmental risk assessment (ERA) of plant protection products (PPPs) in the European Union (EU) does not consider all relevant uncertainties. Critical sources of uncertainties are not included in the currently applied assessment factor (AF) of five, which is used to extrapolate observed effects on single species in the laboratory to expected effects on other species and communities in the field. Several uncertainties are closely related with the properties of test soils (OECD artificial soil or natural soil) in relation to the range of natural soils in the geographical area subjected to the risk assessment. In fact, the risk for soil organisms exposed to chemicals is assessed in the first step based on laboratory tests performed in OECD artificial soil according to the EU Terrestrial Guidance Document. However, OECD artificial soil strongly diverges in its composition from natural soils. Sorption, bioavailability, and the resulting toxicity to non-target organisms depend on the characteristics of the test chemicals and the properties of the respective soil and will, therefore, be different in soils with different properties. In addition, non-target species with different routes of exposure to chemicals will reveal different sensitivities to them. A set of



laboratory experiments was carried out using a targeted combination of five soils (one artificial soil and four natural soils), two test species (*Eisenia andrei* and *Folsomia candida*) and six test substances (insecticides of Cyantraniliprole and Sulfoxaflor, fungicides of Fluazinam and Prochloraz, a herbicide of Prosulfocarb and the chemical metabolite 1,2,4-Triazole) to fill knowledge gaps on uncertainties that have not been adequately addressed by the data available in regulatory databases and the literature. The relationship between the ecotoxicological endpoints for each test substance and the properties of the test soils was evaluated by constraint multivariate analyses (i.e., through Redundancy Analyses) using the EC10 and EC50 values estimated in the laboratory reproduction tests. The results showed that using artificial soil in ecotoxicity testing with soil invertebrates was insufficiently protective, if the current assessment factor of 5 is used. Moreover, analysis of inter-soil variation suggested that any correction factor based solely on organic matter or organic carbon content has limited capability to explain the variation of effects on non-target soil organisms between different soils.

#### **4.06.A.T-02 The Influence of Soil Organic Matter Content on the Toxicity of Pesticides to Soil Invertebrates**

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To account for potential differences in bioavailability (and toxicity) due to different soil organic matter (SOM) contents in natural and artificial soil (AS), in the current European environmental risk assessment (ERA) a correction factor (CF) of 2 is applied to toxicity endpoints for lipophilic pesticides (i.e. log Kow>2) generated from laboratory toxicity tests on soil invertebrates. This CF is based on 14-day earthworm toxicity tests focusing on mortality, and therefore its applicability to other soil invertebrates and toxicity endpoints is questionable. To improve the ecological relevance of ERA approaches, a better understanding of how soil organic matter (SOM) content influences other toxicity endpoints and other soil invertebrates is needed. In the current study, the influence of SOM content on pesticide toxicity to earthworms (*Eisenia andrei*), springtails (*Folsomia candida*), and enchytraeids (*Enchytraeus crypticus*) was investigated. Animals were exposed to five (moderately) persistent pesticides differing in lipophilicity (log Kow 0.6 - 4.7) in four soils (three artificial soils, one natural soil) with different SOM contents (2.5 - 10%), using survival and reproduction as endpoints. The data were used to determine LC50, EC50, and EC10 values, and the relationships between toxicity endpoints and SOM content were analysed through linear regression analysis. The linear regression equations were used to calculate the toxicity in soils containing 10.0% and 5.0% SOM content, and the ratios between these values were used to assess the suitability of the current CF of 2. The results show that pesticide toxicity differed significantly between soils. LC50s and EC50s for all pesticides strongly correlated with SOM content in AS ( $r^2 > 0.82$ ), with pesticide toxicity decreasing with increasing SOM content. In almost all cases, the observed ratios between model-estimated toxicity in the two soils exceeded the CF of 2. Overall, the influence of SOM content on pesticide toxicity differed between pesticides, endpoints, and test organisms, and no clear relationship between toxicity-SOM relationships and lipophilicity was observed. This shows that the CF of 2 is based on incorrect assumptions regarding the relationships between pesticide lipophilicity, SOM content and toxicity and, therefore, may not be appropriate.

#### **4.06.A.T-03 Effect or no effect? The normal operating range of the abundance of soil organisms in ecotoxicological field studies**

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For the registration of PPPs in the EU, ecotoxicological risk assessments for soil organisms are required. Within a tiered approach, laboratory-derived effects on single species are compared to a modelled soil exposure value. In case a risk is indicated, field studies can be required, focusing on recovery of non-target soil organisms within one year after application of the respective product. Although ISO guideline 11268-3 adapted with literature data is used to conduct field studies on earthworms, no OECD test guidelines exists. In addition to lack of guidance for collembola, high natural variability of species abundance may lead to discussions on biological relevance and statistical significance, with a preprogrammed high uncertainty from a regulatory perspective.

Aim of the project was to compile data from EU soil organism field studies to investigate what can be a biologically relevant test item effect, and how to investigate its statistical significance. Therefore, abundance data from 13 collembola field studies from Central Europe was collected. The taxa to focus on were selected by dominance classification according to Engelmann (1978). The normal operating ranges (NORs) were derived based on the mean abundance (individuals/m<sup>2</sup>) for each taxon per sampling point and study as the percentage by which the abundance deviates from the mean control value for a given taxon at one observation point, which can include a certain percentile range (e.g., NOR90 includes all values between the 5<sup>th</sup> and 95<sup>th</sup> percentile; NOR80 all between the 10<sup>th</sup> and 90<sup>th</sup> percentile). The abundance data for test item treatment groups from a given field study were compared to the respective NOR of a taxon and further with the statistical outcome based on different methods like Dunnett, Williams, or the relatively new Closure Principle Computational Approach Test.

Our investigation shows that a high variability in abundance in ecotoxicological field studies is often observed and not uncommon from a biological perspective, but it can be challenging regarding interpretation of potential test item effects. We introduce the NOR concept as a valuable tool to help interpret the results, and to put the outcome of statistical analysis in a

population-relevant context. However, with regards to the high complexity of the data, a field study should be the last step in the RA and not the only ecotoxicological refinement option. Therefore, intermediate tier testing options need to be developed.

#### **4.06.A.T-04 Intermediate Tier Risk Assessment of Plant Protection Products for Soil Invertebrates - Where do we stand?**

*Gregor Ernst, Environmental Safety, Bayer AG - Crop Science Division, Germany*

The conservativeness of the Tier 1 risk assessment of pesticides for soil invertebrates is expected to increase as the upcoming guidance for estimating PEC<sub>soil</sub> will lead to higher exposure estimates and higher failure rates. Further, a new guideline for higher tier studies (earthworm field test) is under development on OECD level, which is expected to lead to an increasing complexity in the higher tier risk assessment. A risk assessment framework should be protective, internally consistent, and cost efficient. However, the upcoming changes described above lead to a situation in which more substances will trigger into a more complex higher tier assessment. Intermediate tier approaches are currently lacking in the European risk assessment framework for soil invertebrates, yet urgently needed. Different intermediate tier options (natural soil testing, non-standard species testing, assessing recovery in laboratory tests, and multi-species tests) were discussed on a workshop in March 2022 in which it was concluded that further work on standardization and risk assessment calibration is needed. Therefore, case studies for *Collembola* were compiled to quantify the relationships between the endpoints of the different study types. The presented case studies allow 1) to compare the recovery pattern of different taxa seen in field studies with those that are predicted from *Folsomia candida* multi-generation studies, and 2) to assess the conservativeness of different intermediate tier risk assessment approaches. The case studies indicate that the recovery predicted in the multi-generation studies with *F. candida* is well in line with the recovery pattern of the sensitive taxa in the field studies. The protectiveness of the proposed intermediate tier approaches is well demonstrated for the available case studies. Reducing the uncertainty in the risk assessment by providing additional experimental evidence must be acknowledged with clearly lower assessment factors (compared to Tier 1) to achieve a cost effective and consistent tiered risk assessment framework.

#### **4.06.A.T-05 Bioaccumulation and mixture toxicity effects of organic pollutants are highly dependent on soil type and test species**

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Soils are mostly contaminated by a mixture of numerous pollutants which enter the environment either by direct (e.g. agriculture) or indirect pathways (e.g. atmospheric deposition). In chemical risk assessment, only single substances in standard soils are tested. However, the uptake and toxicity of substances can be supported by their co-contaminants in a positive (synergistic), additive or a negative way (antagonistic), and also by the properties of the test soils, e.g. clay and organic content.

In this study, the soil invertebrates *Folsomia candida* and *Enchytraeus crypticus* were exposed to chemical mixtures consisting out of two agrochemicals, the fungicide fluazinam and the trisiloxan-based wetting agent Break-Thru S301, and two ubiquitous pollutants, the PAH fluoranthene and the plastic softener DEHP. Substances were applied in binary, ternary and quaternary mixtures with a toxicity of 1 or 2 Toxic Units. The animals were exposed in a 28-day standard reproduction test in which survival and dry weight of the adults were also assessed as well as their bioaccumulation of the substances. Three different field test soils were used: a sandy soil (S), a sandy-organic soil (SO), and a loamy soil (L).

Overall, the chemical mixture was most toxic in the S soil and more toxic to *F. candida* than *E. crypticus*. For *F. candida*, tests with the wetting agent had to be excluded as the high impact on the surface tension skewed results of the reproduction test evaluated by flotation method. However, the remaining data clearly showed that the mixture toxicity effect was additive in S and SO soil, while in L soil it was clearly antagonistic. Bioaccumulation of fluoranthene was clearly highest in S soil and massively increased by the presence of DEHP, followed by SO, while in L soil fluoranthene bioaccumulation completely disappeared in the ternary mixture. For *E. crypticus*, however, the quaternary mixture acted additively in L soil, but antagonistically in S and SO soil.

The study aims to link absolute toxicity of the substances and mixture toxicity effects to tissue and pore water concentrations which are currently assessed. Overall, the results show the clear impact of the test soils and species on bioaccumulation and the manifestation of mixture toxicity effects. Furthermore, it supports the common practice to choose concentration addition as the default or worst-case model for chemical mixtures in the soil environment.

#### **4.06.B Hazards, Risks, and Management of Soil Ecosystems for Sustainable and Environmental Conservation**

##### **4.06.B.T-01 Turning Recovered Street Cleansing Wastes into a Circular Economy Soil Product – A Seasonal Monitoring of Harmful Substances**

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Street sweeping is a routine maintenance operation that collects residues consisting of diverse materials, including litter, grit, leaves, glass, bituminous macadam, paper and plastics and thus may contain potentially harmful elements (PHEs), such as

heavy metals and organic compounds (e.g. total petroleum hydrocarbons, TPHs). Currently, street cleansing residues are treated as waste and have to be disposed of at suitable facilities, e.g. landfill or incineration. However, treatment of street cleansing residues can ensure reclamation (and use) of gravels/aggregates and sands. Finer materials (i.e. silt and clays) are currently disposed of, but have the potential to be used as soil amendments in structural soils and/or as growing media. Due to the decline in soil resources, such material could provide essential qualities in supporting plant life, by also reducing the need for “virgin material” (i.e. dug-up and transported to site) and being used close to its source. Road sweepings are often perceived of as contaminated, however, relatively little is known about the physico-chemical properties of reclaimed street sweeping material, how this varies with time, and what the associated environmental and human health risks are.

This study aims to provide a temporal (over a 12-months period) physico-chemical characterisation of treated and recovered street sweepings (using internationally recognised standard procedures, e.g. British Standards Institution procedures). Our results show consistent physico-chemical properties of the recovered material throughout the study’s period, with metal/elemental concentrations and TPH concentrations, present at concentrations below screening values for use in a public open space. This demonstrates potential capability and suitability for the reclaimed material’s to be used as a soil and/or soil amendment for urban green infrastructure, that may provide a ‘circular’ alternative to ‘virgin’ material and prevent unnecessary landfilling of a useful resource.

#### **4.06.B.T-02 Methodology and Tools to Promote the Reuse of Excavated Soils in France**

*Noémie Dubrac, Samuel Coussy and Virginie Vincenti, French Geological Survey (BRGM), France*

Each year in France, nearly 130 million tons of soil are excavated for infrastructure and development projects. A major portion of this waste is still stored in landfills, whereas they could be used as backfill material or reused as topsoil in many development projects. However, land developers and local authorities consider it risky to reuse these materials, because of the lack of traceability over the flow of excavated soil and because of the lack of soil quality control.

To address these issues, the French government set an ambitious target in 2015 to reuse 70% of construction waste as part of the Energy Transition Law for Green Growth (LTECV) and asked the French Geological Survey (BRGM) to develop a methodology and tools to promote the excavated soil circular economy.

The methodology developed in 2013 and updated since then aims to facilitate, encourage and supervise the reuse of excavated soil while preserving human health and the environment. The last changes, which should be published in the beginning of 2024, will allow soil reuse on surface with different threshold values depending on the site use. For some compounds, bioaccessibility tests will be recommended to better estimate transfers from soils to humans. Additional studies are still permitted if the excavated soil’s quality exceed the threshold values.

This new methodology based on the future use of the site needs to have an efficient traceability to fulfill recent mandatory obligations but also to fulfill environment and human health issues in case of a change of use.

The BRGM developed for the Ministry of the Environment the TERRASS application, a public tool that aims to help public works actors manage excavated soil and make traceability easier. It allows producers and receivers to connect together for soil exchange, and to ensure the traceability of soil flows at different scales thanks to the emission of soils follow-up forms. Since January 2023, the TERRASS application is connected to the French National Register of Soil and Sediments (RNDTS) making it easier to fill in the mandatory declarations established as part of the AGECE law (anti-waste for a circular economy): all soil flows with a volume greater than 500 m<sup>3</sup> must be declared by the producer, sorting/transit facility, and receiver.

The new methodology and regulatory requirements should encourage actors to improve traceability and enhance the circular economy of excavated soil.

#### **4.06.B.T-03 Functional versus compositional tests in the risk assessment of the impacts of chemicals on the soil microbiome**

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In Europe the OECD 216 Soil Nitrogen Transformation Test is used to conduct the risk assessment for the exposure of the soil microbiome to plant protection products. However, this study system has come under criticism for a lack of sensitivity, which has led to calls to further develop the suite of tests available for assessing the risk of PPPs to the soil microbiome, such as the integration of tests of microbial community composition as measured via amplicon sequencing. The proposed use of amplicon sequencing within a standardised regulatory test system brings with it significant challenges, such as the complexities of collapsing data on 1000s of microbial taxa into a single endpoint. Furthermore, it has yet to be established whether the use of more complex, compositional metrics to study the microbiome would provide additional protection to the soil microbiome above and beyond the current functional approaches implemented in OECD 216. Dose response soil nitrogen transformation tests were conducted as per the OECD 216 guideline and at the end of the study period, soils were sampled, and bacterial communities characterised via 16S rRNA amplicon sequencing. Traditional functional endpoints were calculated and compared to a variety of compositional endpoints generated based on microbial diversity, microbial dissimilarity to the control

and threshold indicator analysis. Endpoints generated from the compositional data ranged from approximately 5 times lower to 20 times higher than those calculated using the traditional functional endpoints from the OECD 216 guideline. The endpoints from the compositional analysis spanned an approximately 100-fold range, showcasing a high level of variability between methods of endpoint calculation. Our data show that functional endpoints based on soil nitrogen transformation are in many cases more or at least equally sensitive as endpoints generated from compositional data. Moreover, we found that certain compositional endpoints, such as microbial community diversity and dissimilarity to the control could not consistently detect significant effects that were reported from the functional OECD 216 endpoints. It is our conclusion that amplicon sequencing data does not provide data that would provide additional protection to the soil microbiome above and beyond the current functional approaches implemented in OECD 216. OECD 216 studies may be more sensitive than current opinion suggests.

#### **4.06.B.T-04 Disaggregation behavior in the terrestrial isopod *Porcellionides pruinosus* as a new ecotoxicological endpoint for assessing infochemical disrupting activity**

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Among rapid ecotoxicological tests for screening soil quality, avoidance behavior tests on gregarious edaphic species, such as *Porcellionides pruinosus*, are widely used. However, the effect of soil contamination on the adaptive aggregation ability has not yet been investigated. This research aimed to develop a new ecotoxicological endpoint related to the disaggregation effect for investigating the infochemical disruption at the population level during the avoidance test. This new endpoint was evaluated using tire particles and benzothiazole as preliminary reference substances. As a measure of disaggregation, the Disaggregation Index (*DI*) and the Disaggregation Groups (*DG*) are presented to quantify the effect of contaminants on the aggregation behavior. The disruption of aggregation in a group of ten individuals is evaluated alongside the sub-lethal avoidance test after 48 hours. The extent of disaggregation is measured by the number of subgroups formed. The *DI* and *DG* indexes span from 0 to 1, representing respectively the highest degree of aggregation and disaggregation achieved during the test. Our findings reveal that all woodlice successfully passed the avoidance test validation but failed to exhibit a gregarious behavior, indicating a fragmentation within the population. These results propose combining avoidance behavior with disaggregation in individuals of *P. pruinosus*. The concentration observed to induce alteration in gregariousness is in the range of the sublethal effects inducing avoidance. Considering both aspects can produce more accurate and robust environmental risk assessment results.

#### **4.06.B.T-05 Importance of Heat Stress in the Risk Assessment of Pesticides for Soil Arthropods**

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The pressure from anthropogenic pollution of soils, such as agricultural pesticides, is currently addressed in pro- and retrospective risk assessments as outlined in the Regulation (EC) No 1107/2009. The framework for prospective ecological risk assessment is mainly based on hazard assessments from laboratory tests in which environmental conditions are maintained at optimum for the organism in focus. Given that environmental factors (e.g., temperature) can influence the toxicokinetics and toxicodynamics of chemicals, we propose that laboratory-based hazard assessment should include important abiotic factors.

Human-caused climate change is already affecting many weather and climate extremes in every region across the globe. The global rise in temperatures poses a significant threat to soil-dwelling organisms, unsettling the delicate balance of ecosystems. Nature is directly affected by the rising mean temperature and the ubiquitously projected increased frequency and intensity of heat waves. Recent studies highlighted alterations of uptake and elimination of toxicants in soil invertebrates due to temperature induced variation in metabolic rates and diffusivity. Nevertheless, our understanding of the repercussions of high temperature on the performance of soil arthropods, especially regarding sub-optimal high temperatures and their influence on pesticide uptake and effects, remains largely unexplored.

Here, we aim to address this lack of knowledge with a testing approach, combining a commonly used springtail (*Folsomia candida*) reproduction test with incubation at a range of non-optimum high temperatures. As our test compound we used the widely used fungicide, fluazinam. The test also included analysis of internal concentration as well as metabolites of fluazinam. Concentrations of fluazinam and its metabolites in spiked soil and in springtail bodies were analysed using a newly developed LC-MS/MS method. Preliminary results revealed that high environmentally realistic temperatures hamper detoxification processes in *F. candida*. Furthermore, we found an increase of metabolites over time, and with the increase of temperature, indicating that detoxification processes are significantly influenced by high temperature as reported by others. In the ongoing work, we investigate the interaction between sub-optimal temperature and fluazinam effects using a full factorial test design.

#### **4.06.P Hazards, Risks, and Management of Soil Ecosystems for Sustainable and Environmental Conservation**

##### **4.06.P-We328 Predicting Earthworm Toxicity Endpoints in Non-Standard Soils: Risk Assessment Concept and Model Validation**

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Earthworm risk assessment for plant protection products (PPP) in Europe is normally based on toxicity tests performed in artificial test soils with either 5% or 10% organic matter (OM). Soil characteristics, in particular organic matter content, influence the bioavailability of chemicals, and one would therefore expect different levels of toxicity in natural soils. Here we present a concept for predicting toxicity endpoints in untested soils using the results of a toxicity test (EC<sub>50</sub> or LC<sub>50</sub>) in a standard soil containing 10% OM and a previously published empirical model. The model predicts the uptake and elimination rate constants of a one-compartment toxicokinetic model based on earthworm species properties (lipid content and specific surface area), topological polar surface area of the molecule, and the organic matter content of the soil. We evaluate the accuracy of the model in predicting EC<sub>50</sub> and LC<sub>50</sub>, using previously published toxicity data on 22 organic compounds that were tested in multiple soils with different organic matter content. The model showed high accuracy with 87% of predictions within a factor of 2 of the observations. The predictions are overall conservative, tending towards somewhat lower endpoints than those empirically determined in non-standard test soils. Based on the observed accuracy, we conclude that the model is suitable for predicting toxicity endpoints in untested soils. Incorporating such an approach into the ecological risk assessment for earthworms in Europe allows an increased level of ecological realism and provides an opportunity to refine the PPP risk assessment according to the soil properties of concern.

#### 4.06.P-We329 Novel Strategy to Assess the Risk of Sequential Application of Tank Mixtures to Non-Target Soil Organisms

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Mixing two or more plant protection products within the sprayer equipment's tank (tank mixtures; TMs) is a cost-effective method, but has been neglected by European Union's legal directives. Moreover, most ecotoxicological studies on PPP mixtures have evaluated only the impact of binary combinations on non-target soil organisms, failing to mirror real-world application scenarios, besides using only one test organism. Aiming to contribute to filling this gap, the present study proposes a novel strategy to evaluate the risk of a sequential application of TM to non-target soil invertebrates. The adequacy of the proposed strategy was evaluated by the comparison of the effects on the reproduction of non-target species to the single application of TMs with the cumulative effects on reproduction of the same species over sequential applications of the same TMs, as it occurs in a real scenario usually adopted in soybean crops. Reproduction tests were carried out using the species *Eisenia andrei*, *Folsomia candida* and *Hypoaspis aculeifer* following ISO and OECD guidelines. A natural soil collected in an agricultural field was used as test soil. Ranges of increasing concentrations of TMs usually applied in soybean crops were used in ecotoxicological tests. Test concentrations of TMs were based on toxic units estimated with effect data obtained from the literature. Test treatments were prepared from a stock solution with the mixture of products in a proportion related to their individual recommended doses. The sequential experiment followed field application sequences, including microbial inoculation for degradation. Results showed varying sensitivities among organisms in response to different TMs. For sequential applications, earthworms showed the highest sensitivity in TM1+TM2, while predatory mites after the application of TM3. In contrast, single applications showed different patterns, with collembolans been the most sensitive organism in TM2, TM3, and TM4, while *E. andrei* was the least sensitive for TM1, TM2 and TM3, and *H. aculeifer* for TM4. Data produced show that in a real sequential application scenario there is a cumulative effect of the PPP applications that considerably increases the risk of PPPs to non-target soil organisms. The strategy proposed in the present study seems to allow an adequate assessment of the cumulative effect of PPP applications and therefore, has potential to be considered in ecological risk assessment of PPPs in the future.

#### 4.06.P-We330 Why do we neglect key drivers for soil health in environmental risk assessment? – A plea for the use of nematodes as bioindicators

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Nematodes constitute the most abundant animal phylum on earth, with up to 10 million organisms inhabiting one square meter of soil. Due to their high taxonomic and functional diversity, they occupy all trophic levels in soil food webs, making them key drivers for soil health. Although plant parasitic nematodes are among the most severe threats for agriculture, their free-living species are essential for the maintenance of important soil functions, such as nutrient cycling. In the meanwhile, standardized and innovative methods with a high level of preciseness are available to assess effects of chemicals on nematodes (ISO 10872, small scale microcosms). Moreover, due to their high densities, nematodes offer many advantages in field studies (easy sampling, small sample volumes, statistical power, etc.). Although also EFSA already mentioned nematodes as key drivers of soil functioning (EFSA 2017, EFSA Journal, 15(2), 225; <https://doi.org/10.2903/j.efsa.2017.4690>), this organism group is still not adequately represented as bioindicators in the risk assessment of soil contaminants, such as plant protection products. This can mainly be explained by two assumptions: (1) Nematodes are not sensitive to toxic chemicals, such as pesticides. (2) Nematodes are a challenging organism group in terms of taxonomic identification, due to their small size and morphological similarity, resulting in a low number of experts and, thus, hampering the use as bioindicators. With this presentation, we want to bring light in the discussion about nematodes in environmental risk assessment and to get rid of some tenacious myths about the low suitability of nematodes as bioindicators. As there are simply too few studies on effects of chemicals (especially pesticides) on soil nematodes, a valid comparison of sensitivities to other soil organisms, such as springtails and earthworms is not possible. However, using standardized tools, such as the soil toxicity test with *Caenorhabditis elegans* (ISO 10872), or

innovative microcosm tests, it could be revealed that nematodes are comparatively sensitive to certain compound classes, such as fungicides. Moreover, in the light of innovative techniques of DNA-based taxonomy, the argument of morphological challenges with nematodes has to be put into a new perspective. More studies, including available innovative tools, are urgently needed for reliably evaluate the suitability of nematodes as bioindicators of soil health.

#### **4.06.P-We331 Targeting Validity Criteria in Natural Soil Testing as Intermediate-Tier Approach with Earthworms, Soil Mites and Springtails**

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Natural soil laboratory testing as an intermediate-tier option for soil organisms would be a valuable supplementation for the current environmental risk assessment because there is a vast gap between laboratory testing in artificial soil and complex field studies. Major technical challenges for natural soil testing are the selection of suitable soils regarding representativity and the achievement of control validity criteria to obtain reliable results.

The influence of the soil parameters soil moisture, pH, sand, silt, clay and organic matter on the validity criteria number of juveniles and mortality was investigated for *Eisenia fetida*, *Hypoaspis aculeifer* and *Folsomia candida* following the guidelines OECD 222, OECD 226 and OECD 232. The influence of different moisture levels on number of juveniles and mortality was investigated by testing a range of soil water potentials in OECD artificial soil and a natural soil for each species from nearly water saturation (pF 1.3 / -2 kPa) to permanent wilting point of plants (pF 4.2 / -1.5 MPa). In separate testings, the influence of the remaining soil parameters was tested with all three species in four natural soils covering a wide range of soil properties representative for soils under agricultural use, using a soil water potential within the ecological optimum of the respective species.

The results show that the guideline validity criteria for artificial soil are also achievable in typical agricultural soils. Soil parameters like pH, texture or organic matter content play a subordinate role in explaining differences in control performance. The dominating factor influencing control reproduction of earthworms and springtails is not the water content itself, but the soil water potential (negative pore water pressure) that determines the bioavailable water.

Laboratory testing with natural soils according to the current guidelines is feasible with some technical adaptations. Natural soil testing will help reducing uncertainties in bioavailability of substances in comparison to OECD artificial soil, providing a more realistic scenario.

#### **4.06.P-We332 Molecular-ecological Studies on Bile Acid Degrading Bacteria in Soil for Assessment of Plant Protection Products**

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One of the important functions of microorganisms in soil is the metabolic transformation of organic material. This function can be affected by the introduction of chemicals such as plant protection products (PPP) into the environment and may thus have a negative impact on the fitness of soil organisms. The metabolic capacity of soil bacteria has, so far, been mainly determined by the sum parameter nitrification. However, these tests do not provide information on changes in microbial communities occurring after the introduction of such substances into soil. There is an evident knowledge gap between the function and the structure of microbial soil communities. To close this knowledge gap, specific carbon and energy sources that are used as substrates by defined taxa only, could be used to correlate metabolic activity with the structure of the microbial soil community. In this respect, bile acids might serve as appropriate substrates. Bile acids can be used by a limited number of bacterial genera such as *Comamonas*, *Pseudomonas* or *Rhodococcus*. Therefore, microbial metabolism of bile acids might be established as an alternative way to evaluate the metabolic capacity of soil samples in response to the introduction of chemicals.

During the first stage of the project, the abundance of bile acid degrading bacteria from soil spiked with bile acids was analyzed with colony forming units (CFU). The degradation of bile acids was analyzed with HPLC-MS measurements. Additionally, amplicon sequencing of the genes encoding the 16S rRNA was conducted to analyze the microbiome. In a second step, the effect of the PPP propamocarb and thiram was evaluated in a soil test. Soil samples were taken and incubated with bile acids. By comparing soils with and without PPP, we will evaluate if a reduced bile acid degradation correlates with reduced bacterial abundance (CFU and qPCR) of the specific bile acid degrading taxa.

Based on the available data that the genera *Comamonas* and *Pseudomonas* belong to the few known bile-acid degrading taxa, their increased abundance in bile-acid containing soils supports the suitability to consider this metabolic trait for linking metabolic capacity to the structure of bacterial soil communities. By adding PPP to the soil, ongoing tests will reveal whether the function of bile acid degradation is influenced and whether this influence is reflected by the structure of the microbial community in the soil.

#### **4.06.P-We333 Effects of Heavy Metals from Artisanal Gold Mining and Pesticides on Farmland Soil Quality and Earthworm Biodiversity in Batouri, East Cameroon**

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Batouri gold deposits in the East Region of Cameroon have long been subjected to Artisanal and Small-scale Mining (ASM) activities resulting in pollution, land degradation and deforestation. The progressive loss of agricultural land to mining activities necessitates action to preserve the soil quality of remaining farmlands for continued productivity. Earthworms are a major component of soil fauna communities and are highly susceptible to pollutants. Their number and diversity are an important indicator of soil quality. This research set out to assess the effects of heavy metals from Artisanal and Small-scale Gold Mining (ASGM) and pesticides use on soil quality and earthworm diversity in farmlands in the Batouri gold mining district. Surface soil samples were collected from 6 farmlands located at varying distances (5m to 1765m) from mining sites and analyzed for physicochemical characteristics, Cd, Cu, Fe, and Hg. Questionnaires were used to determine pesticides use by farmers. Earthworm diversity and abundance were assessed using quadrats. Results showed that the Hg concentrations at all sites exceeded the European Environment Agency guideline in agricultural soils leading to extremely high soil contamination factor ( $23.6 \leq Cf \leq 114$ ) and potential ecological risk ( $944 \leq Er \leq 4575$ ). Hg contributed between 96% and 99.6% to the overall potential ecological risk index (RI). The geo-accumulation index (Igeo) indicated that anthropogenic activities was the source of Hg enrichment in the soil. It also indicated low to moderate Cu pollution from anthropogenic sources, most likely from pesticides. 8 earthworm species from the three major ecological categories were identified (3 epigeic, 4 anecic and 1 endogeic species). Earthworm abundance followed the order Endogeic > anecic > epigeic species. Principal Component Analysis showed a positive association of earthworm abundance with the % organic matter, % silt, Cu concentration and the distance away from the mining locations while the Cf, Er and Igeo for Hg were negatively correlated to the earthworm abundance. ASGM is therefore causing widespread Hg pollution in Batouri and significantly affecting the soil quality and earthworm abundance in farmland soils. In addition, the use of pesticides containing Cu may be causing moderate soil pollution. There is therefore the need for risk assessment and management of Hg and Cu in farmland soils in Batouri to prevent ecological and human health effects.

#### **4.06.P-We334 Ecotoxicological characterization of nano-biochar obtained by ball-milling**

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Biochar has garnered extensive research attention due to its potential applications in agriculture, soil improvement, and contaminated area remediation. Its capacity to enhance soil fertility, water and nutrient retention, and positive impact on soil microorganisms make it a promising agricultural tool. However, concerns about the safety of biochar application persist, as it can contain both organic (e.g., PAHs) and inorganic contaminants (e.g., heavy metals).

Recent focus has shifted to nano-biochars (n-BCs), produced by ball milling bulk biochars (b-BCs). This method, considered eco-friendly, cost-effective, and reliable, offers advantages such as fine powder production and suitability for milling toxic materials. N-BCs, being produced from waste materials, have a reduced environmental footprint and show potential for diverse applications, making them efficient and adaptable.

The study aimed to assess the influence of n-BCs (<100 nm) and their bulk counterparts, prepared from various feedstocks and pyrolysis conditions, on PAH content and distribution. Ecotoxicological tests with *Aliivibrio fischeri*, *Folsomia candida*, and *Daphnia magna* evaluated potential environmental risks.

Ball milling's impact on biochar particle size significantly affected PAH content. After ball milling, the total content of  $\Sigma 16$  PAHs in selected n-BCs decreased by 10.8% to 59.2%. Similarly, freely dissolved  $\Sigma 16$  PAHs in n-BCs decreased from 10.1% to 65.1%. Changes in the contribution of Ctotal and Cfree PAHs based on the number of rings were also observed in all n-BCs.

Ecotoxicological tests revealed higher toxicity to *A. fischeri* for n-BCs compared to b-BCs, with toxicity increasing over time. In contrast, *F. candida* and *D. magna* tests showed no mortality or immobilization but inhibited reproduction in both b-BCs and n-BCs.

In conclusion, the study highlights the impact of ball milling on n-BCs' properties, influencing PAH content and ecotoxicological effects. N-BCs show promise, but careful consideration of their environmental impact and potential risks is necessary for widespread applications.

#### **4.06.P-We335 An agricultural systems' perspective on soil and biodiversity: Learnings from a set of field trials with soil health and climate balance in a broader sustainability context**

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To guide and implement long-term improvements at the interface between agriculture, soil health and biodiversity, the use of

suitable metrics and a perspective on sustainability performance can be decisive, ensuring economic, ecological and social viability of the proposed systems.

In the case of this work a broader sustainability setup was chosen for field trials with the aim to create a more comprehensive perspective on agricultural production and its various challenges for farmers, including potential trade-offs and unintended consequences. Among other parameters, specific focus was set on a number of soil health metrics in different production systems to learn more about the interaction between soil organic carbon, nutrient balance, earthworm abundance, greenhouse gas emissions at the interface to impacts on yield, crop rotation and production costs. Above-ground carabid populations were investigated as a proxy for biodiversity for both the in-field and off-field area.

Results from the first two years have been presented at the previous SETAC and this work aims to complement and draw conclusions on key learnings and how to approach these themes in the future.

To increase the sustainability of agriculture a transparent perspective on performance and trade-offs is required. Such a perspective will be decisive to not only enable realistic reference frames and protection goals for the ecotoxicological assessment of plant protection products but also to create a broader perspective for life cycle assessments and practical improvement of sustainability performance at farm level. The next steps would be to explore how to minimize the trade-offs and therefore increase the sustainability of the applied practices and enable upscaling.

#### **4.06.P-We336 Co-occurrence of Di-2-ethylhexyl phthalate (DEHP) and Titanium Dioxide Nanoparticles (nTiO<sub>2</sub>) in Soil Aggravates Ecotoxicity Associated with Disrupted Energy Budget in Nematode *Caenorhabditis elegans***

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Emerging contaminants, such as the plasticizer di(2-ethylhexyl) phthalate (DEHP) and nanomaterial titanium dioxide nanoparticles (nTiO<sub>2</sub>), pose potential threats to soil-dwelling communities. Understanding the singular and joint toxicities of these contaminants is crucial for predicting their environmental impact. We investigated the effects of DEHP and nTiO<sub>2</sub> on the soil nematode *Caenorhabditis elegans* (*C. elegans*), using concentrations ranging from 1 to 100 mg/kg. Our results indicate that both DEHP and nTiO<sub>2</sub> individually caused significant reproductive impairments in *C. elegans*, with the lowest observed adverse effect levels (LOAELs) of 100 and 1 mg/kg, respectively. For combined exposure, the effects of 1 mg/kg DEHP and nTiO<sub>2</sub> (1, 10, and 100 mg/kg) were examined. We found that combined exposure to DEHP and nTiO<sub>2</sub> resulted in aggravated toxicity, revealing LOAELs of 100 and 1 mg/kg nTiO<sub>2</sub> for growth and reproduction, respectively. To unravel the physiological modes of action (pMoAs), we employed a dynamic energy budget (DEB) model. The model allowed us to derive predicted no-effect concentrations and elucidate the heightened reproductive costs associated with individual DEHP or nTiO<sub>2</sub> exposure. The results indicate that the combined presence of DEHP and nTiO<sub>2</sub> increased the cost of growth and reproduction. Considering that energy budgets are linked to energy status, we assessed adenosine triphosphate (ATP) levels in *C. elegans*. Singular exposure to DEHP (1 mg/kg) or nTiO<sub>2</sub> (100 mg/kg) decreased ATP levels. Notably, combined exposure synergistically exacerbated the impact on ATP levels, suggesting a lower energy threshold for joint exposure. Furthermore, mitochondrial damage and reactive oxygen species (ROS) levels were also evaluated. While singular nTiO<sub>2</sub> exposure increased worms with mitochondrial damage, co-exposure to DEHP and nTiO<sub>2</sub> further heightened damage by 200%. Additionally, the combined exposure significantly elevated ROS levels. Our study highlights that joint exposure to DEHP and nTiO<sub>2</sub> results in heightened toxicity in soil. Furthermore, we propose an adverse outcome pathway involving disruption of energy allocations, linking molecular events to declines in organismal propagation and, potentially, population decline. This research contributes valuable insights into the complex interactions of soil co-contaminants and their impact on soil-dwelling organisms.

#### **4.06.P-We337 Field Study with Carbendazim in Brazil to Evaluate Effects on Local Earthworm Community**

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Risk associated to the application of plant protection products (PPPs) in soil invertebrates is usually defined by considering its toxicity to standard species as surrogate/representative of specific key organism groups. Previous studies have reported that local earthworm species may be more sensitive to specific PPPs than the standard species *Eisenia andrei/fetida*. Because of that, countries outside Europe, such as Brazil, have highlighted the necessity and the interest of adapting the ecological risk assessment (ERA) of PPPs to their reality (i.e. taking into account local species). However, data regarding the effect of PPPs on earthworm local communities in countries of Latin America (LA) are scarce and the high local endemism of earthworm species and the restricted number of species with broad distribution in countries like Brazil have made the adaptability of soil ERA difficult to achieve. Therefore, field studies are needed to check sensitivity of natural earthworm communities under local environmental conditions. These data are crucial to understand at which point, data from European field studies can be used in ERA of LA countries. Moreover, local field studies will help in identifying possible technical limitations (e.g. population densities and seasonal abundances may condition the definition of adequate sampling dates), to calibrate lower tier risk



assessment and to validate effect models for LA scenarios. Therefore, a field study was conducted in a grassland area in Santa Catarina State, Brazil, aiming to 1) compare the sensitivity of earthworm field populations with those from European studies; 2) calibrate Brazilian lower tier risk assessment. A commercial formulation of Carbendazim was used as test substance and a randomized block design with three carbendazim doses (0.32, 1.8 and 10 kg a.s./ha) plus a control was followed. Six earthworm species belonging to four families (Glossoscolecidae, Ocnerodrilidae, Megascolecidae and Lumbricidae) were identified in the pre-sampling and showed a higher earthworm density compared to the sampling after 1 month. A heterogeneous distribution of earthworms was found, independently of the application rates. Samplings evidenced that earthworms density is highly influenced by soil moisture and recent rain events. Data from samplings before and 1, 6 and 12 months after Carbendazim application will be presented and discussed and technical limitations will be pointed out.

#### **4.06.P-We338 "Evaluation of soil enzyme activities in abandoned mine area"**

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As commonly known, mining sites were highly contaminated of heavy metals. Soil enzymes play a major role in the cycling process of soil and plant. Also, it can be used as an indicators of soil condition. In this study, the soil enzyme activity of contaminated soil was assessed. Three heavy metal contaminated site (S1 – S3) were selected, including reference site soil (R). Five types of soil enzyme activity were evaluated such as acid phosphatase activity (ASA), arylsulfatase activity (ASA),  $\beta$ -glucosidase activity ( $\beta$ GGA), fluorescein diacetate hydrolase activity (FDA), and Protease activity (PRT), using 1 g of each site soils. The total heavy metal content was higher in the order of site 1 (S1), site 2 (S2), site 3 (S3) and R. Site 1 soil showed a significant decrease in the activity of all types of soil enzymes. As a result, heavy metals contents can inhibit the soil enzyme activities such as acid phosphatase activity, arylsulfatase activity,  $\beta$ -glucosidase activity, fluorescein diacetate hydrolase activity and protease activities. Soil conditions are related to microorganisms and soil interactions, therefore evaluation of soil enzyme activity can provide a major information. *Acknowledgement-This work was supported by Korea Environment Industry & Technology Institute (KEITI) funded by Korea Ministry of Environment (MOE) 2022002450002(RS-2022-KE002074).*

#### **4.06.P-We339 From invasion to solution: Optimizing a Sargasso Biochar Strategy for Chlordecone Sequestration that not alter Caribbean Soil Quality**

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The use of biochar's (BC) as a rehabilitation tool for polluted soils is increasingly being studied, especially in the case of the Caribbean chlordecone (CLD) crisis. BC have the ability to sequester pollutants but may also have an impact on soil fertility, especially on the microbial communities, biological fertility keystone. The effect of BC on soil microorganisms is very diverse and remain incompletely investigated. The impact of BC on soil seems to be the most impactful during the maturation period of the soil-BC mixture. This phase allows the BC to interact with the pollutant without external competition granting an optimal sequestration. This phase, varies from one study to another, especially du to BC and soil diversity. So, it is necessary to determine the optimal maturation time for each case.

This study aims was to pinpoint the optimal maturation period of Sargasso (invasive algae) BC for efficient CLD sequestration in Caribbean soils and to elude its impact on microbial communities' structure. For this, CLD assays and 16S rDNA quantification in 2 contaminated soils (Andosol and Nitisol) amended or not with 2% of Sargasso BC were carried out during 5 months.

For Andosol 50% reduction of CLD were reached after 24 hours and seems to reach a 90% plateau reduction after 29 days. For Nitisol, at 24 hours the CLD reduction was up to 37% and 50% of CLD reduction was reached at 5 months. The difference between the two soils may be explained by the large difference in initial soil contamination (Andosol: 1.85 mg/kg, Nitisol: 7.99 mg/kg). A high CLD concentration implies a possible BC surface saturation as well fewer CLD-BC bonds, contributing to a slowdown of sequestration. Andosol is known as a matrix strongly sequestering CLD, so it would have been logical to obtain shorter ripening times for Nitisol. So, these results highlight the strong correlation between the optimal maturation time and the initial level of contamination. Preliminary measurements on the samples were made to visualize the evolution of bacteria abundance in soil revealed by 16S rDNA copy number. No statistically differences were measured between the control and the BC treatment at each kinetic time. These results tend to demonstrate that for Caribbean soil the Sargasso BC does not alter bacteria abundance. Within this framework, additional analyses are underway aiming to effect of BC amendment on the structure and diversity of bacterial communities by metabarcoding approach.

#### **4.06.P-We340 Can the land use affect the risk for inducing antibiotic resistance by heavy metals?**

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The ongoing spread of antibiotic resistance is a global challenge for human health. Soils, due to their high microbial diversity, act as an important reservoir of microbial resistance to antibiotics. There is growing concern that heavy metals (HM) can co-select for antibiotic resistance in soil. This study aimed to investigate microbial tolerance to HM and antibiotics in different land uses and the sensitivity of antibiotic resistance induction to further contamination. Agricultural and forest soils with varying levels of HM contamination were sampled and analyzed. Replicated microcosms were set up and spiked with Cu or tetracycline, or used as a control. Microbial biomass, respiration, growth, community composition, and tolerance to Cu and

tetracycline were measured over several weeks. The results showed that bacterial tolerance to Cu and tetracycline varied among different land uses. Bacterial Cu tolerance was highest in contaminated forest soil, intermediate in pristine forest soil, and lowest in agricultural soils. On the other hand, tetracycline tolerance was highest in agricultural soils, intermediate in pristine forest soils, and lowest in contaminated forest soils. This suggests that bacterial tolerance to antibiotics was linked to land use rather than only the HM pollution level. The inducibility of bacterial Cu tolerance was higher in pristine forest soil compared to contaminated forest soil, potentially due to differences in initial levels of microbial species diversity. Additionally, the development of tetracycline tolerance in agricultural soil may be limited by microbial biodiversity. The speciation of tetracycline, influenced by the pH of the medium, was found to be more toxic to bacteria in contaminated forest soils compared to pristine soils. Overall, our study highlights the complexity of microbial tolerance to HM and antibiotics in different ecosystems and the need for further research in this area.

#### **4.06.P-We341 Assessment of pesticide mixtures on soil organisms: challenges for non-standard endpoints**

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Agricultural application of pesticides often occurs as combination of several products, either together in a tank mixture or in a series by applying similar or different products to the same crop during a vegetation period. The consideration of mixture effects in the environmental risk assessment has repeatedly been called for in the last decades, and it has been demonstrated frequently that existing prediction models such as Concentration Addition (CA) as well as Independent Action (IA) would be fit for purpose in many cases. These cases include e.g. the effects of a mixture of substances applied together and to be assessed for acute effects in a standard test organisms. However, the assessment of mixture effects with regard to non-standard or higher-tier endpoints, such as soil microbial community composition and/or long-term effects in field studies, are still lacking guidance and supporting evidence. Similarly, potential mixture effects of serial applications, in field or laboratory studies, are rarely investigated and difficult to assess with the available mixture toxicity models. Here, we combine the experience gained in several studies with soil microorganism communities and soil invertebrates as well as evidence from the literature to discuss the possibilities of a meaningful mixture assessment for these complicated, but realistic mixture scenarios.

#### **4.06.P-We342 Ecotoxicity of the monoterpene eugenol on soil microbial communities**

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Terpenoids, a diverse class of natural compounds found in plants and some microorganisms, play pivotal roles in various biological processes. Among them, eugenol stands out, known for the wide-ranging pharmacological activities, exhibits potent anti-inflammatory, antiseptic, and antimicrobial properties. Due to its increasing use, an environmental toxicity assessment is warranted.

This work evaluates the ecotoxicity of eugenol on soil microbial communities obtained from an uncontaminated crop field in Zaragoza, Spain, at different doses (0, 1, 10, 100, and 1000 µg/mL). The overall ability of natural ecosystems to break down 31 typical carbon sources was indicated by the Average Well Colour Development (AWCD), which was determined by Biolog EcoPlates® analysis. At 0, 1, 10, and 100 µg/mL, the results showed a steady decrease in AWCD and metabolite intake, leading to complete growth inhibition at 1000 µg/mL. Interestingly, there was a decreased capacity to metabolize carbon sources, most notably carbohydrates and polymers.

As a result, this study shows that an increase in eugenol consumption could have an impact on the environment. These findings improve our knowledge of how eugenol affects soil community structures, providing valuable insights for proactive environmental management strategies.

#### **4.06.P-We343 Intermediate Tier Testing Alternatives for Ground Invertebrates – A Granular Insecticide Case Study**

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Soil organisms such as beetles or collembolans represent some of the most abundant communities in agricultural fields and surrounding areas and are non-target organisms of concern in terms of potential exposure to plant protection products (PPPs). While the standard risk assessment for non-target arthropods includes various intermediate testing options – extended laboratory studies with fresh/aged residues – the risk assessment for PPPs for soil macro- and meso-fauna is based on Tier 1 tests and, when unacceptable risks are identified, higher tier testing consisting only of field studies is required. Therefore, as there is a lack of intermediate levels from standard lab tests to full field effect studies, achieving more realistic exposure conditions in laboratory tests is deemed a necessary and practical step providing additional information in refined risk assessments of certain PPPs.

In this case, an alternative design was developed for two soil organisms exposed to a granular insecticide formulation: the rove beetle *Alleochara billineata* exposed to fresh and aged residues (Grimm *et al.*, 2000) and the collembola *Folsomia candida* (OECD 232) exposed to fresh residues. In the standard method, a test item is mixed within the soil substrate homogeneously, while the alternative method simulates the intended field application of the product *i.e.* as granules are to be applied in the field at 4 cm deep furrows, the test design was adapted to create a layer of granules at 4 cm depth in the test units. While this design

doesn't fully mimic the field application in furrows, it represents a worst-case approach where organisms can avoid the granules in the majority of the arena.

For *A. billineata* a standard aged residue study (granules mixed within soil homogeneously) resulted in 90% mortality at 159 days aged residues (application rate – 1.39 kg a.s./ha), while the modified study design (granules buried 4 cm deep), resulted in 2.1% mortality with fresh residues (application rate – 1.16 kg a.s./ha).

For *F. candida*, the modified exposure design with granules buried 4 cm deep resulted in a reduction in reproduction of 9.6% and 28% at 1xGAP (10 kg PPP/ha) and 5xGAP (50 kg PPP/ha), respectively.

This technique enabled organisms to avoid direct granule exposure, demonstrating more realistic effects when mimicking field application. The alternative study design may form the basis for intermediate tier testing for ground invertebrates when assessing granular formulations.

#### **4.06.P-We344 A multispecies test system as tool for an intermediate test system in soil ecotoxicology and risk assessment**

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For the registration of PPPs in the EU, a tiered ecotoxicological risk assessment (RA) for soil organisms is required. In the recent framework endpoints obtained from standardized Tier 1 laboratory studies exposed in artificial soil are compared to a modelled predicted environmental concentration. As higher Tier only the performance of a field study is foreseen, where potential effects on soil organism communities are evaluated over one year. This knowledge gap between Tier 1 laboratory studies and the field study can be filled with results from intermediate tier test systems. A laboratory multi species test design could be a valuable methodological approach for such an intermediate tier test system.

We designed a laboratory multispecies test design with different settings to answer relevant questions concerning inter- and intraspecific competition, feasibility and reliability of the test system and the results. The basic test design is adapted from the recent OECD guidelines (OECD 226 and OECD 232) for testing single soil arthropod species. The test medium is a defaunated natural agricultural soil, where different combinations of soil taxa were added. The tested collembolan species were *Proistoma minuta*, *Sinella curviseta* and *Folsomia candida*. As predator the gamasid mite *Hypoaspis aculeifer* was included. Further included species are *Panagrellus redivivus* (Nematoda) and *Enchytraeus albidus* (Enchytraeidae). Beside different controls, boric acid and chlorpyrifos-methyl were tested as chemical stressors.

The test design can be used to draw conclusions about direct toxic effects of the chemical stressors, effects based on competition for food resources as well as predatory effects. The statistical outcome of the test results and adaptations of the test system to obtain better results are also discussed.

#### **4.06.P-We345 Representativeness of Standard Species of Microarthropods for Pesticides Risk Assessment in Brazil**

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Microarthropod species have been used as bioindicators of soil quality, being very sensitive to abiotic and biotic changes in ecosystems, as well as to soil contamination. Although these organisms represent a small proportion of soil biomass and respiration, they have a crucial role in nutrient cycling and microbiological regulation in soil ecosystems. To improve the pesticide risk assessment in Brazil, this work aimed: 1) to list the representative collembola and predatory mite species from Brazilian agricultural areas; 2) to compare the sensitivity of the standard species of collembola, *Folsomia candida*, and the predatory mite *Hypoaspis aculeifer*, with alternative species, in ecotoxicity tests with natural and artificial soil. To achieve the first objective, a literature review was performed. The most common sub-order was Entomobryomorpha, with emphasis on the Entomobryidae and Isotomidae families (50% of all). Although *F. candida* was not found in Brazil, the genus was present in agricultural fields. The literature review showed that *F. candida* is very sensitive to different pesticides, but there is a lack of data for comparisons among species. To achieve the second objective, ecotoxicity tests were performed with the standard species *F. candida* and *H. aculeifer*, and the alternative collembola species *Proisotoma minuta* and *Sinella curviseta*, in one natural tropical soil (Oxisol) and one tropical artificial soil (TAS), to answer the questions: a) Are the results from the sensitivity of *F. candida* and *H. aculeifer* enough to protect other species?; b) Are the pesticide effects in artificial soil similar to that in Oxisol? For this purpose, the active ingredients (a.i.) from the two most commercialized pesticides in Brazil were evaluated, imidacloprid and mancozeb. Reproduction tests followed the ISO 11267, with adaptations for the alternative species (e.g., 20 organisms per replicate for species with sexual reproduction). NOEC and EC values were compared among species and soils for each a.i. Results indicated that *F. candida* is potentially enough sensitive to protect other Collembola species and

that, depending on the species, protection values obtained with natural soil can be more restrictive than those obtained with artificial soil. Additional studies with other microarthropod species and their exposure are of crucial importance to better understand the reliability of extrapolating results obtained with *F. candida* to protect other species.

#### **4.06.P-We346 Compatibility of commercial bacterial consortium of plant biostimulant with copper-based pesticides**

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Plant-biostimulants based on beneficial microorganisms (mPB) are currently being developed to boost crop productivity mainly by increasing nutrient efficiency in soil, representing an environmentally friendly alternative to conventional agrochemicals. Several reports have indicated that these nature-based products increase the ability of plants to grow under adverse environmental conditions, such as drought and low temperatures. However, studies reporting the compatibility of these microbial-based biostimulants with agrochemicals commonly used in agriculture are scarce and not fully understood. To address this knowledge gap, our study aimed to investigate whether commercial microbial based-biostimulants containing a consortium of bacteria, namely *Azospirillum brasilense* and *Pseudomonas fluorescens*, could lead to synergistic, antagonistic, or neutral effects on plant performance and soil functioning when applied in combination with conventional and nano-copper agrochemicals. For this, inhibition of biostimulant activity was tested in the presence of agrochemicals, using microdilution assays and enumeration of colony-forming bacteria. The inhibition assays were established using three doses of microbial biostimulants ( $3 \times 10^6$  (recommended dose);  $6 \times 10^6$ ; and  $3 \times 10^7$  cell/ml), exposed to a wide range of concentrations of three formulations of commercial pesticides with active ingredients of copper sulfate, copper oxychloride, and copper hydroxide, and one nano-based pesticide (nCuO): 0 to 30 mg[Cu]/ml. A pot experiment was conducted using a natural soil inoculated with microbial biostimulant, in the presence of *Medicago sativa* L. and exposed to copper-based agrochemicals, for 28 days, to validate the compatibility of these products. As a result, microbial-based biostimulants were susceptible to conventional- and nano-pesticides in a dose-dependent manner, in which the highest inhibition rate was observed in the highest concentrations of copper. The preliminary results from soil pot experiments suggested that the microbial biostimulant stimulates plant growth, dependent on the copper concentration tested. Thus, this study emphasizes the potential use of this bacterial consortium of plant-biostimulants in conventional agricultural systems. Furthermore, this data can also be considered a baseline for an in-depth investigation of the functional recovery of degraded soils affected by intensive agricultural practices.

#### **4.06.P-We347 Integrating Terrestrial Model Ecosystems into Soil Risk Assessment – A Tier 3 Refinement Option**

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Soil dwellers are an essential part of healthy soils that support ecosystem functions and services such as nutrient cycling essential for ensuring crop growth (EFSA 2017). In the European Union (EU), there is the requirement to assess and understand the risk of plant protection products (PPP) to soil dwelling organisms. The current risk assessment for soil organisms compares the NOEC (No Observed Effect Concentration) and/or EC10/20 (Effect Concentration with a 10% / 20% effect) to the maximum Predicted Environmental Concentration in the soil (PECsoil) calculated according to FOCUS (FOCUS 1997).

The proposed introduction of a new exposure assessment procedure for in-field soil organisms is expected to impact the current risk acceptability of many PPPs. Specifically, Tier 1 and 2 of the EFSA (2017) exposure assessment procedure uses the Persistence in Soil Analytical Model (PERSAM), which integrates European scale environmental and agronomic data for calculation of PECsoil values for the three EU Regulatory Zones and Individual Member States. Therefore, existing PECsoil values (FOCUS) are subject to change, and it is also likely that the new PECsoil values (PERSAM) will be higher than current ecotoxicological endpoints, causing PPP risk assessments to fail.

Where a compound fails the risk assessment at Tier 1 of the ecotoxicological risk assessment, field studies are often the only option for endpoint refinement. Intermediate tiers such as extended laboratory studies (Tier 2: non-standard species, exposure refinement, modelling) and soil mesocosm studies (Tier 3) to address issues in the risk assessment are rarely conducted. This lack of regulatory accepted interim tiers necessitates an increased need for the development of robust intermediate tier options to close this growing gap in soil risk assessment. Terrestrial Model Ecosystems (TMEs), where soil cores taken from a pasture or meadow to ensure sufficient abundance and biodiversity for reliable outcomes, have been shown to be a robust testing system, however, they have yet to be incorporated into soil risk assessments. As soil risk assessments aim to protect in-field soil dwellers, TMEs represent a worst-case scenario and results can be regarded as protective of in-field organisms, in a similar concept to aquatic mesocosms representing edge-of-field systems.

Here we aim to present ideas and concepts on how to use TMEs as a refinement option at Tier 3 for the effects on soil dwelling organisms.

#### **4.06.P-We348 Ecotoxicity of commercial nanopesticide to the soil model *Enchytraeus crypticus* (Oligochaeta): from standard tests to long(er)-term effects assessment**

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Nanopesticides hold the promise of being more environmentally sustainable alternatives to conventional pesticides due to their increased effectiveness and targeted delivery, allowing the reduction of application rates, hence increasing agricultural productivity in a more sustainable way. However, given its novelty, the environmental risk assessment of these advanced materials is mostly absent, particularly concerning non-target organism. Among those, soil living invertebrates are in the first line of exposure from pesticides application and their run-offs. Thus, the aim of the present study was to investigate the toxicity of a commercial insecticide, with reported nanofeatures - Karate Zeon®, and compare it to the active substance lambda-cyhalothrin, for the soil model invertebrate species *Enchytraeus crypticus* (Oligochaeta). Effects were assessed in LUFA 2.2 soil, covering various endpoints and life-stages, beyond the standard, from short- to long(er)-term exposures. Four test types were performed (endpoints:days): avoidance test (avoidance behaviour: 2 days), OECD standard reproduction test (survival, reproduction plus adults' size: 28days) and its extension (total number organisms: 56 days), and Full Life Cycle (FLC) test (hatching and juveniles' size: 13 days; survival, reproduction and adults' size: 46 days). Results showed that enchytraeids did not avoid Karate Zeon® nor the active substance lambda-cyhalothrin, which could be due to neurotoxicity. Karate Zeon® was equally toxic to the active substance lambda-cyhalothrin in *E. crypticus* and there was no indication that toxicity would increase in prolonged exposure (based on the 56d results). FLCt results indicated that juvenile life stage was the most sensitive life stage to both Karate Zeon® and lambda-cyhalothrin exposure, resulting in an increased toxicity for the survival of adult animals that were exposed from the cocoon stage (as opposed to the exposure during adult stage alone). The similar toxicity between Karate Zeon® and lambda-cyhalothrin shows that, if given at a lower application rate, the nanopesticide could still theoretically pose lower risks to the enchytraeid population and hence it would represent an improved benefit to substitute to an advanced material. We here show the potential of novel approaches to test methods, beyond the standard, where the longer-term exposure and additional endpoints represent remarkable added value to the interpretation.

#### **4.06.P-We349 “FORESEE”: A Spatial and Temporal Explicit (DEB-)TKTD Model for Earthworms**

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Earthworms represent an important factor for soil health as they are main contributors to nutrient availability in soil and influence the soil structure due to their burrowing behavior. For these ecosystem services, they are used in risk assessment of plant protection products as non-target organisms. To reduce the need of laborious field studies and to be able to predict the behavior or population dynamics of different earthworm species based on different weather and/or exposure scenarios, Johnston et al. introduced two independent individual based earthworm models in 2014 and 2018, the “EEEworm”, for *Aporrectodea caliginosa* and *Lumbricus terrestris*, respectively.

With the development of the dynamic energy budget model (DEB) and extension of the AddmyPet-database in the past years, the possibility was provided to convert the previous earthworm models into the generic earthworm model “FORESEE” with the individual development of the worms based on DEB theory. Moreover, to add to realism, species-dependent movement due to moisture and food gradients calibrated to lab experiments as presented in Gergs et al. 2022 was newly integrated into the model.

To evaluate the performance of the new model, we will use the case studies applied by Johnston et al. 2014 & 2018 and compare the predictions of FORESEE and the previous work of Johnston et al. on which FORESEE is based on.

#### **4.06.P-We350 Long-term nanoplastics exposure affects soil carbon dioxide emission and microbial carbon metabolism in agricultural soil**

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Nanoplastics (NPs), arising from physical breakdown or chemical degradation of plastic pollutants, can accumulate in soil through sludge application and landfill. With the escalating production and disposal of plastic, the environmental concentration of NPs is likely to increase, posing potential risks to soil ecosystems. However, the harmful effects of NPs on the soil function and carbon metabolism remain much unclear. In this study, we investigated the impacts of NPs on agricultural soil and its microbial community. Our findings showed that, following a 56-d incubation, the NPs-treated soil exhibited higher total organic carbon compared to the control. At day 56, the activities of extracellular enzymes, glucosidase, and xylosidase were significantly inhibited in the NPs-treated soil. Moreover, carbon dioxide (CO<sub>2</sub>) emission was found to notably decrease in soil in the presence of NPs after 56 days. We also observed a significant decrease in the abundance of the most prevalent bacterial species in the soil samples (*Bradyrhizobium* sp.). In addition, the genes associated with carbon metabolism were altered by the presence of NPs. Our study indicates that long-term exposure to NPs (56 days) hinders carbon metabolism and reduces CO<sub>2</sub> emissions in the agricultural soil samples, potentially linked to the diminished abundance of *Bradyrhizobium* sp.

#### 4.06.P-We351 Ecotoxicological effects of roadside soils on earthworms - the role of pollution variation with distance and habitat type

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Earthworms are one of the animals with the largest biomass in soil, playing critical roles in improving the permeability, water retention of the soil and improving soil quality. As a model organism, earthworms are more sensitive to pollutants than other soil organisms. The aim of the study was to evaluate the toxicity of contaminated highway soil to earthworm *Dendrobaena veneta*. The study sites – forest and meadow are located near the highway in Poland. Soil from the study sites was collected at different distances from the highway of the non-forest (meadow) site. The negative impact of soil collected at non-forest site to earthworms' survival increased with the distance from the traffic line. The effect of contaminated soil was dependent on the distance from the road. In the soil collected 25 m from the traffic line, mortality was insignificant and no change in mortality was detected with increasing exposure time, while at 50 m from the traffic line, a significant lethal effect was detected from the first weeks of exposure and increased with increasing exposure for time. The effect of contaminated soil on earthworm weight depended on habitat type. The lowest weight loss was in the soil collected at the traffic line in the non-forest site. Weight loss decreased with increasing distance from the traffic line, with significantly lower weights compared to soil-exposed earthworms collected 25 m from the traffic line.

#### 4.06.P-We352 Long-chain Hydrocarbon-Degrading Bacterial Communities in Long-Term Polluted Soil in Ogoniland, Niger Delta

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This study is the first to identify long-chain n-alkane degrading genes in a Niger Delta soil microbiome severely impacted by oil pollution. Soil samples were collected from different depths, namely surface level (SPS) and one meter below the surface (SPSS), along with an unpolluted control sample (UPS) from a site approximately 1 km away. Physicochemical attributes, soil microbiome, and isolated bacterial cultures were characterized through standardized analytical methods, Illumina MiSeq and Sanger sequencing. Isolates were further screened for *almA* and *ladA* genes, which encode enzymes essential for hydrocarbon degradation. The study employed various enrichment media, including Hexadecane (HDX), Paraffin oil (POL), paraffin wax (PWX), and heavy bonny crude oil (HCO), and assessed degradation capabilities using the 2, 6-dichlorophenol indophenol (DCPIP) redox indicator. Total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) were substantially higher in polluted soils. A range of bacterial species, including *Marinomonas* and *Pseudomonas*, exhibited significant hydrocarbon degradation capabilities. Only *Marinomonas* possessed the *almA* gene. Targeted amplicon analysis identified *Koribacteraceae*, *Rhizobiaceae*, and *Streptococcaceae* as core bacterial families in polluted soils. Alpha diversity metrics revealed higher microbial diversity at the surface soil compared to subsurface and highlighted the richest diversity in the unpolluted soil. The findings suggest that indigenous bacterial communities in the polluted site have the potential for long-chain hydrocarbon degradation, offering promising avenues for bioremediation strategies.

#### 4.06.P-We353 Effects of Temperature and Moisture on the Ecotoxicity of Metal-Based Fungicides Towards Earthworms

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The aim of this study was to determine if varying soil moisture contents and different temperatures will influence the toxicity of single and binary mixtures of the fungicides, copper oxychloride and mancozeb, (in different concentrations) towards earthworms (*Eisenia andrei*) as bioindicators of soil quality. Tests were done using artificial soil in accordance with the OECD guidelines under two different temperatures viz. 20°C and 25°C and two different moisture contents viz. 30% and 50%. The concentrations for copper oxychloride (CuOx) were 200, 500 and 1000 mg/kg and mancozeb (MnZn) 44, 850 and 1250 mg/kg in single and binary mixtures. Endpoints measured included metal analyses, comet assays (DNA analysis) and soil enzymatic analyses. DNA damage increased as the concentrations of the treatments increased. Significant differences (p<0.05) were found between the different temperatures and soil moistures although the different experiments produced different results. Body metal analysis test corresponded with the comet assay test wherein there was an increase in body metal concentration as the concentrations of the treatments increased (p<0.05). Significant differences (p<0.05) were found in the copper and manganese concentrations although no significant differences were found in the zinc concentrations. Soil enzymatic analysis indicated significant differences in the alkaline phosphatase and glucosidase tests for the single and binary (copper oxychloride and mancozeb) treatments and at the 25°C 50% exposure it was the most significant. It is evident that fluctuating temperatures and soil moistures had varying effects on the single and binary mixtures of copper oxychloride and mancozeb. It was concluded that varying temperatures and soil moistures would have a significantly different result on the effects of metal-based fungicides towards earthworms.

**Keywords:** Alkaline phosphatase; Body metal analysis; Climate change; comet assay; Copper oxychloride; *Eisenia andrei*; Glucosidase; Mancozeb; Moisture; Temperature

#### 4.06.P-We354 Heat Waves Decrease the Avoidance of *Folsomia candida* to the Fungicide Fontelis 20 SC

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With increased mean temperature, heat waves, drought, cold snaps and extreme rain, climatic extremes cause severe changes in the environment and challenge biodiversity both above and below ground. Since the soil fauna is an important pillar in most soil ecosystem services, studying the effects of climate-induced stressors on these is essential. Extreme climate events can presumably also alter the effects of plant protection products on soil organisms. Hence, our focus is on the effects caused by the interaction of heat waves, drought, and agrochemicals in the model organism *Folsomia candida* (Collembola).

Here, our test substance is Fontelis 20 SC, a frequently used fungicide in the EU. The active ingredient penthiopyrad has not yet been tested on soil fauna and has limited data on its toxicity to other organisms. Thus, we studied the single and combined effects of heat waves, drought, and Fontelis 20 SC on the avoidance behaviour of *F. candida* (ISO 17512-2:2011). Based on observations in other tests with heat waves, we hypothesized that heat and drought change the avoidance behaviour towards Fontelis 20 SC compared to standard conditions.

Our treatment groups were one of three concentrations of Fontelis SC (750, 187.5, and 46.875 mg penthiopyrad/ kg dry soil) on one side, offered against clean soil, and a control group with clean soil on both sides. These concentrations were combined with the following 4 environments: Standard temperature (20 °C) with 50% and 35% of water holding capacity (WHC); heatwave (24 °C for 24 h then 28 °C for 48 h) with 50% and 35% WHC. We introduced twenty animals into each jar, with ten replicates for each treatment. We analysed the avoidance in each environment by a paired t-test and the combined effects of all stressors on the avoidance with a generalized linear model.

First results suggest that Fontelis 20 SC induces avoidance in high concentrations and preferential behaviour in low concentrations in *F. candida*, while the heat wave prevents overall movement. This suggests grave consequences for Collembola in contaminated sites during heat waves. In conclusion, this study highlights the importance of integrating climate change into ecotoxicological studies, not only as a potential stressor but also as a synergistic stressor in combination with agrochemicals.

#### **4.06.P-We355 Natural Soil Testing in the OECD 226 Soil Mite Reproduction Test using Dimethoate**

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Soil pollution is a global issue that must be effectively addressed using appropriate risk assessment methodologies. Current laboratory testing for ecotoxicological risk assessment (ERA) of plant protection products (PPP) for non-target soil organisms using OECD artificial soil does not reflect the high variation in chemical-physical soil properties found in natural agroecosystems. Using natural soils for laboratory testing as an intermediate-tier option for soil organisms would be a valuable supplementation for the ERA.

Soil mites are regarded as crucial biological markers to evaluate possible effects of PPP, because as predators they add a new trophic level to the community of soil arthropods. However, for the OECD test species *H. aculeifer*, the usage of natural soils in laboratory testings are not adequately investigated, yet.

Four typical agricultural soils were compared to OECD artificial soil following OECD 226, to investigate if specific soil parameters have an influence on *H. aculeifer* lethal and reproduction toxicity of dimethoate.

We found that all tested soils were suitable for *H. aculeifer*. In case of biodegradable dimethoate, soil microbial biomass - the total mass of soil microbial degraders - was beside clay and organic matter one of the main explanatory factors for different toxicities between the tested soils. OECD artificial soil ranged among the soils with higher toxicity and lowest EC50 (3.75 (3.51-4.00) mg/kg sdw) and the lowest toxicity and highest EC50 (9.52 (8.10-11.19) mg/kg sdw) was found in a loamy soil with high microbial biomass.

#### **4.06.P-We356 Ecotoxicological effects of sugarcane vinasse in natura and biogested in methanogenic reactor on the reproduction of Enchytraeus crypticus**

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Vinasse is a subproduct of the sugar or ethanol industry that has been applied in crops as a fertilizer, thus preventing the high contamination of direct discharge in water bodies. Although fertilizer use is considered a circular economy aggregating value to this subproduct, it is important to understand if the continued recycling of vinasse as a fertilizer is also safe and sustainable for the soil environment. This study evaluated if sugarcane vinasse causes any adverse effect on an important soil model organism, the potworm *Enchytraeus crypticus*. The tests were carried out for 21 days of duration using sugarcane vinasse in natura (collected from a large-scale sugar and ethanol plant located in Pradópolis in the State of São Paulo, Brazil) and sugarcane vinasse biodigested (effluent of a lab-scale (2.1L) methanogenic anaerobic structured bed reactor (AnSTBR) from the Biologic Process Laboratory (LPB) in a methanogenic reactor) and LUFA 2.2 soil, and were exposed to six vinasse

concentrations (0.23, 0.92, 3.59, 14.38, 57.50 and 230 ml/kg dry soil, the highest concentration corresponding with the recommended field dose). The concentration of vinasse did not affect the survival or reproduction of the enchytraeids ( $p = 0.273$  and  $0.489$ , Two Way ANOVA, factor A). The type of vinasse (in natura or biodigested) did significantly impact the ecotoxicological response ( $p < 0.05$ , Two-Way ANOVA, factor B), with higher survival and reproduction in the exposures to biodigested sugarcane vinasse. Such an increase was, however, also observed in the controls. Our results indicate that in natura sugarcane vinasse can alter the chemical characteristics of the soil but has a low impact on the life cycle of *E. crypticus*. It is recommended to expand the understanding of the potential effects of the continued use of sugarcane vinasse for other species or soil organism, and also to assess its effects on aquatic organisms considering the potential runoff occurring in the field.

#### **4.06.P-We357 Abundance of frequently found soil organisms (Collembola) in ecotoxicological field studies in Central Europe and determination of their normal operating range – part 1**

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For the registration of PPPs in the EU, an ecotoxicological risk assessment (RA) for soil organisms is required. In that context, field studies are conducted if a potential risk to non-target soil organisms cannot be excluded. They focus on recovery of earthworms or Collembola within one year after application of the respective product on grassland or arable soil. However, no official OECD Test Guidelines have been adopted for such field studies; ISO guideline 11268-3 adapted with literature data is used to conduct field studies on earthworms. Collembola field studies are generally conducted following these recommendations, yet there is a lack of guidance for evaluation and interpretation of results. Especially where a high natural variability of abundance of respective taxa is observed, this may lead to discussions on biological relevance and statistical significance, with a preprogrammed high uncertainty on the outcome from a regulatory perspective.

We analysed and reviewed historical control data to investigate natural variability in Collembola populations in field studies conducted on grassland or arable soil in Central Europe. Frequently occurring species per study were selected based on the dominance classification of Engelmann from 1975. Variability in species abundance for selected taxa was investigated within one study, and across different studies, and the normal operating range (NOR) was determined. Due to the lack of a specific NOR definition for soil organisms in the literature, we propose to define it as the percentage by which the abundance deviates from the mean control value for a given taxon at one observation point, which can include a certain percentile range (e.g., all values between the 5<sup>th</sup> and 95<sup>th</sup> percentile).

Based on this evaluation, abundance of a species in treated samples can be compared with the NOR of the control for a specific study. The results shall support data interpretation of field studies and help to distinguish between natural variability and biologically relevant test item effects.

In the first part of this poster, the frequently found species in soil cores and pitfall traps of the available soil and grassland studies are presented. Based on our investigation, *Parisotoma notabilis* and *Protaphorura armata* are frequently found in soil cores of arable soil studies, while *Lepidocyrtus cyaneus* and *Isotoma viridis* are more prominent in grassland studies.

#### **4.06.P-We358 Abundance of frequently found soil organisms (Collembola) in ecotoxicological field studies in Central Europe and determination of their normal operating range – part 2**

*Agnes Schimera*<sup>1</sup>, *Jörg Hanisch*<sup>2</sup>, *Gregor Ernst*<sup>3</sup>, *Zhenglei Gao*<sup>4</sup>, *Michael Thomas Marx*<sup>5</sup>, *Frank Staab*<sup>6</sup>, *Pernille Thorbek*<sup>7</sup> and *Lijuan Yan*<sup>8</sup>, (1)ADAMA Deutschland GmbH, Germany, (2)Rifcon GmbH, Germany, (3)Environmental Safety, Bayer AG - Crop Science Division, Germany, (4)Data Science, Bayer AG, Germany, (5)Bayer AG, Germany, (6)BASF SE, Germany, (7)Apd/EE, BASF plc, United Kingdom, (8)BASF Services Europe GmbH, Germany

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Based on this evaluation, abundance of a species in treated samples can be compared with the NOR of the control for a specific study. The results shall support data interpretation of field studies and help to distinguish between natural variability and biologically relevant test item effects.

In the second part of this poster, the derived NORs of selected frequently found species from individual studies are presented, and compared with other studies where these species occur. Data shows that an overall NOR for a certain species and season cannot be derived; the NOR should be determined for each species and study individually. For a clear definition of the NOR, specific protection goals are required.

#### **4.06.P-We359 Misfortunes never come singly: Interplay of temperature and soil moisture on toxicity of Cu contamination in springtails, *Folsomia candida***

*Jian Ge<sup>1</sup>, Stine Slotsbo<sup>1</sup>, Jesper Givskov Sørensen<sup>2</sup>, Christian Frølund Damgaard<sup>1</sup>, Alex Robinson<sup>3</sup>, Emily Eagles<sup>3</sup>, David Spurgeon<sup>3</sup> and Martin Holmstrup<sup>1</sup>, (1)Department of Ecoscience, Aarhus University, Denmark, (2)Department of Biology, Aarhus University, Denmark, (3)UK Centre for Ecology & Hydrology (UKCEH), United Kingdom*

Ecotoxicologists have been endeavouring to incorporate important climatic stressors into environmental risk assessment (ERA) of anthropogenic contaminants. Temperature has been widely studied and recognised as one of the most important climatic factors influencing chemical effects on ectotherms. For soil-living arthropods, soil moisture has also been reported as a critical environmental factor not only for their survival, growth and reproduction but also for how they respond to simultaneous existence of contaminants. In this study, we conducted a full factorial experiment investigating the effects of temperature, soil moisture and Cu contamination on a typical soil arthropod, *Folsomia candida*. We hypothesize that the interplay of suboptimal temperatures and moisture conditions increases Cu bioaccumulation and consequently reduces physiological performance of springtails *F. candida*. Soils contaminated with three copper levels (approx. 40, 400 and 1500 mg/kg dry weight soil) were collected from a former timber preservation industry at Hygum, Denmark. Age-synchronized springtails (12-14 d; ten individuals per replicate) were exposed to the three exposure levels in Hygum soil and distributed to combinations of 5 temperatures (5, 10, 20, 24 and 28 °C) and 6 soil moisture levels (soil water potential: -3, -5, -10, -40, -70 and -100 kPa) with 5 replicates. After a 28-day exposure, adult springtails were weighed, and the numbers of adults and juveniles were counted to calculate survival, growth of adults and reproduction. The internal Cu concentration of springtails was measured using Inductively Coupled Plasma Mass Spectrometry. This research demonstrated synergistic interactions between suboptimal temperature, drought and Cu contamination reducing the survival, growth and reproduction of springtails, which shed new light on the role of multiple climatic stressors on soil arthropods under heavy metal contamination. Our findings also call for consideration of climatic stressors in existing ERA policies.

#### **4.06.P-We360 Evaluating the Effect of Carbendazim on Soil Fauna Feeding Activity through a Bait-lamina Test**

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This study investigated the effects of the application of a fungicidal formulation with Carbendazim as active substance on the feeding activity of soil fauna using the Bait-lamina test following the procedures described in ISO 18311. A field test was conducted in a natural grassland and pasture area in Santa Catarina State, Brazil, in Nitisol with no history of pesticide application in the last 10 years. A Randomized Block Design (RBD) comprising three Carbendazim doses (0.32, 1.8 and 10 kg a.s./ha) plus a control (only sprayed with water) was followed adopting the recommendations of ISO 11268-3 for earthworm field test. Twenty-four bait-lamina sticks (i.e. 3 groups of 8 bait-lamina) were introduced in each plot 33, 188, and 365 days after fungicide application and left in the field each for 15 days. After this period, the percentage of bait consumption was estimated in each plot and at the three sampling times. The percentage of consumptions were compared between treatments by a repeated measures ANOVA followed by Dunnett test ( $p < 0.05$ ) for comparisons with control. In the 33-days sampling, significant differences were observed in the feeding activity between test treatments. The 0.32 and 1.8 kg a.s./ha treatments showed significantly higher consumptions compared to control, with mean consumptions of 62.5%, 52.1% and 33.8%, respectively for 0.32, 1.8 kg a.s./ha and control treatments. On the other hand, the highest dose of 10 kg a.s./ha showed a consumption of 38.7%, which was not significantly different from control. The significantly higher consumption found in bait-lamina of 0.32 and 1.8 kg a.s./kg treatments could be related to direct and/or indirect effects of an increase of microbial activity resulting from the degradation of Carbendazim molecules, which most likely does not happen at excessively high doses as 10 kg a.s./kg. At 188- and 365-days samplings, no significant differences were detected in consumptions of bait-lamina between treatments compared to control. These results suggest that Carbendazim does not negatively affect feeding activity of soil fauna in this soil type. However, measured actual soil concentrations of Carbendazim (residue analysis is ongoing) and the comparison of bait-lamina consumption data with soil fauna community measurements (in terms of diversity and abundances) would be desirable to confirm the non-effect of Carbendazim we found through bait-lamina.

#### **4.06.P-We361 Sustainable agriculture strategies in the face of Climate Change: Biosolid compost and trace elements impact on soil and crops**

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Increasing soil organic carbon stocks can strengthen resilience of rainfed Mediterranean crops against shifting rainfall patterns. Biosolid compost (BC) offers an affordable and broadly available solution for these agricultural soils, yet its use may unintentionally amass trace elements (TE) in the soil. Long-term field studies have revealed notable TE buildup in the soil due to repeated compost applications. For this purpose, bioavailable fractions of harmful TE should be considered, given that these fractions are easily uptaken by plants and can be transported by drainage water. Additionally, numerous uncertainties exist concerning the dynamics of TE within BC under climate change conditions. Thus, changes in soil hydrology could accelerate or reduce the release and lixiviation of TE from soils. This research aims to understand TE inputs through compost, their behavior under drought conditions, and their impact on crop accumulation. A factorial experiment with 6 plots (6 × 50 m) was established under Mediterranean conditions. Each plot was divided into two subplots; half receiving no amendment (control) and the others treated with 50 T/ha of BC. Rainout shelters simulated reduced rainfall were established at each subplot. A crop rotation of legumes and wheat was selected. Analysis of the compost complied with regional legislation for TE content. Soil samples taken immediately after compost addition (November 2022, T=0) and before crop harvest in April 2023 (T=1) exhibited significantly higher total TE content in amended soils at T=0. The lower pH in amended soil heightened TE availability, a trend sustained for some TE (As, Cd, Cu, and Ni) at T=1, although at very low levels (µg kg<sup>-1</sup>), despite pH adjustment. After 5 months of study, reduced rainfall at T=1 did not notably affect TE availability. Crop shoot TE levels (*Vicia faba*) across all treatments remained within normal ranges without signs of phytotoxicity. Continuous monitoring is necessary, as excessive compost application over time could escalate TE availability, potentially impacting crops in the long run.

#### **4.06.P-We362 Sustainable waste management: The impact of olive pomace-derived Entomofertilizer on soil health**

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Integrating olive pomace into insect-based substrates presents a novel approach to addressing waste management challenges in the olive oil industry while increasing the sustainability of the use of insects for food and feed. The dual benefits of this approach, effective waste management and the production of high-quality and sustainable protein, align with global efforts towards a circular bioeconomy and sustainable food systems, making it a highly relevant topic in environmental and agricultural research.

*Hermetia illucens* (black soldier fly BSF) can use various organic substrates and produce frass, which is proposed as a valuable entomofertilizer. The efficacy of olive pomace-based entomofertilizer OP-BSF<sub>frass</sub>, particularly its non-phytotoxic nature despite being derived from olive pomace, has been demonstrated in plant growth assays. However, further research is necessary to understand better its impact on soil microbiota and related functions.

This study aims to evaluate the effects of frass on soil quality parameters by looking at microbial community responses to the presence of (OP-BSF<sub>frass</sub>) using soil functional parameters related to enzymatic activities (urease, β-glucosidase, aryl sulfatase, dehydrogenase, phosphatases-acidic and alkaline) and community-level physiological profiling (CLPP). OP-BSF<sub>frass</sub> was added to Lufa 2.2 standard soil in different concentrations (from 0.3 to 9.6 % w/w) and incubated at 20°C for sixty two days. Results demonstrated that soil enzymatic activity increases in a dose-dependent manner with the concentration of OP-BSF<sub>frass</sub>, stimulating β-glucosidase, urease, alkaline phosphatase, and at the lowest concentration of frass, aryl sulphatase, and acidic phosphatase. Also, a significant decrease in the activity of acidic phosphatase, aryl-sulphatase, and dehydrogenase was observed in the highest concentrations of OP-BSF<sub>frass</sub> (4.8%, 9.6%). The CLLP was analyzed using the average-well color development (AWCD) index, demonstrating a significant increase in the carbon-utilization pattern in microbial communities present in OP-BSF<sub>frass</sub> treatments compared to the control soil. Overall, the results reveal that the moderate application of OP-BSF<sub>frass</sub> stimulates the nutrient cycling and the metabolic activity of microbial communities, potentially due to the nutritional decomposition of the frass. The promising results of this entomofertilizer, derived from waste-fed *H. illucens*, are crucial in advancing sustainable waste management.

#### **4.06.P-We363 Soil fertilization with a novel Zn fertilizer: The effects of Zn-Al-NO<sub>3</sub> layered double hydroxide on soil biological and chemical properties and maize development**

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Zinc is an essential micronutrient that is vital in several plant physiological functions. When its supply is inadequate, the development of plants can be adversely impacted. To overcome this problem, especially in Zn-deficient soils, fertilizers containing Zn are applied. Still, their (over)application has been associated with harmful consequences to the environment,

such as soil degradation, through runoff causing water eutrophication, among others. In this sense, it is critical to develop novel Zn fertilizers to sustain crop production and quality, while maintaining the health of ecosystems. Layered double hydroxides (LDHs) are multifunctional nanomaterials whose potential for agricultural application as an alternative to Zn fertilizers has been progressively explored. Yet, to ensure sustainability, it is extremely important to understand their behaviour, fate, and toxicity in ecosystems. In this context, the aim of the present study was to evaluate the potential of Zn-Al-NO<sub>3</sub> LDH as an alternative Zn fertilizer, at relevant concentrations for agronomic applications. Zinc sulphate was tested as a conventional fertilizer. This integrated assessment lasted 19 days and combined the evaluation of plant performance and Zn bioaccumulation in maize (*Zea mays*), with soil quality indicators and Zn fate assessments. The ecotoxicological assessment evaluated the seed emergence and growth of maize and the functionality of soil microbial communities, through the activity of soil enzymes. Additionally, the bioavailability and accumulation of Zn in maize root and shoot tissues and the contents of Zn and nitrate in leachates were characterized. Applying Zn-Al-NO<sub>3</sub> LDH to soil slightly increased the shoot length and biomass of maize and the content of Zn in the plant roots. Soil enzymatic activities were influenced by both Zn-Al-NO<sub>3</sub> LDH concentrations in soil and incubation time, in an enzyme-specific response. The activity of soil enzymes increased over time in all treatments, except for urease. The soil treatment with Zn-Al-NO<sub>3</sub> LDH did not change the concentration of Zn in the DTPA-fraction and in leachates, the content of nitrates increased with the Zn-Al-NO<sub>3</sub> LDH increasing concentrations. Overall, our work shows that Zn-Al-NO<sub>3</sub> LDH can be an effective alternative to conventional fertilizers.

#### **4.06.P-We364 From waste to resource: Exploring the use of Black Soldier Fly entomofertilizers to replace mineral fertilizers in agriculture practices**

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The interest in sustainable agriculture increased the exploration of new organic fertilizers, such as insect frass, a by-product of insect farming constituted by the leftover fecal matter. This study explores sustainable alternatives to traditional mineral fertilizers by assessing the efficacy of *Hermetia illucens* (black soldier fly) larvae frass, in agricultural applications. The effects of frass from different sources was tested in a soil pot experiment with *Brassica rapa*, where it was tested: (1) a reference frass produced by the insect digestion of agricultural leftovers and (2) a frass produced from the insect digestion of olive pomace, a residue from the olive oil industry known for its phytotoxic properties. We aim to assess the potential of both frasses to complement or replace conventional mineral fertilizer, NPK (nitrogen, phosphorus, potassium). Experimental treatments include NPK alone, each frass alone (at relevant application rates based on nitrogen content), and in combinations of each frass and NPK (at different proportions). The experimental control group consists of the natural LUF 2.2 soil, the same soil used in the treatments, but without adding fertilizers. With this experimental setup, we selected plant performance and soil functional parameter endpoints for the efficacy of test treatments. Germination, biomass (growth, weight) and productivity endpoints (seeds, pods) of *B. rapa* under these different fertilization regimes were assessed following OECD 208 and ISO 22030-2011 guidelines, also ensuring the safety of using olive pomace-derived frass. Enzymatic activities (urease,  $\beta$ -glucosidase, aryl sulfatase, dehydrogenase, and both acidic and alkaline phosphatases) were measured to assess the impact of the treatments on soil biochemical properties and nutrient cycling. Our results suggest that both frasses stimulate plant growth, potentially offering a viable strategy to reduce the dependence on mineral fertilizers. Olive pomace frass does not exhibit phytotoxic effects on *B. rapa*, indicating the bioconversion process's effectiveness and providing input in using insects in waste management. Furthermore, soil enzymatic activity results reveal a positive influence of frass treatments on soil health, with positive implications for more environmentally friendly and resource-efficient farming methods.

#### **4.06.P-We365 Ecotoxicological Evaluation of Drilling Cuttings from Oil Wells for Organomineral Fertilizer Development: Predicting Impacts on Forest Ecosystems**

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The oil industry holds significant importance for the Brazilian economy, being a major contributor to non-renewable energy raw materials. However, it also generates substantial waste, including liquid, solid, and gaseous residues with significant pollutant potential. Among these, gravel from oil well drilling stands out due to its pollution potential and large volume. Research and studies have focused on this waste due to its chemical components such as K, Ca, P, Mg, Fe, among others, making it potentially useful in non-food agricultural sectors, forestry, and the restoration of degraded areas as organomineral fertilizer. However, its use is limited due to high sodium (Na) content, which can be ecotoxic to plants and soil invertebrates. As a strategy, gravel from oil wells was treated to reduce Na content. This study aimed to evaluate the ecotoxicity of gravel from post-salt and pre-salt oil well drilling after treatment, analyzing its potential for organomineral fertilizer production and recommending its use in forest areas. Two ecotoxicity tests were conducted with treated gravel from pre-salt and post-salt layers. The first assay, based on ABNT NBR/ISO 11269-2 and adapted by Silva (2019), assessed growth and biomass with the tree species *Mimosa scabrella*. Different concentrations (0.25% to 5%) of pre-salt and post-salt gravel in Tropical Artificial

Soil (SAT) were tested, evaluating aerial and root length, as well as dry mass. The second test, a multispecies test used Tropical Artificial Soil with gravel concentrations (0.25% to 5%) to assess the response of two test organisms, *Folsomia candida* and *Enchytraeus crypticus*. It examined if contaminants affected soil fauna feeding activity and tested organism reproduction. Data were analyzed by comparing treatments to the control (no gravel addition) using Analysis of Variance (ANOVA) followed by Dunnett's test ( $p < 0.05$ ) or Kruskal-Wallis test followed by Dunn's test. Overall, results showed no ecotoxicity associated with the waste for tree species germination and initial development. In the multispecies test, different concentrations of treated gravel did not adversely affect the feeding activity or reproduction of test organisms. Concentrations ranging from 0.25% (equivalent to 6.5 tons/ha) to 5% (equivalent to 130 tons/ha) demonstrate potential for use as organomineral fertilizer, especially in forest planting and degraded area restoration scenarios.

#### **4.06.P-We366 A Spatiotemporally Explicit Modeling Approach for Exposure and Risk Assessment of Off-field Soil Organisms**

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Natural and semi-natural habitats of soil living organisms in cultivated landscapes can be subject to unintended exposure by active substances of pesticides used in adjacent fields. Spray-drift deposition and runoff are considered major exposure routes into such off-field areas. In this work we develop a model (xOffFieldSoil) and associated scenarios to estimate exposure of off-field soil habitats at flexible levels of realism (<https://github.com/xlandscape/xOffFieldSoilRisk>). The modular approach consists of components each addressing a specific aspect of exposure processes, e.g., pesticide use, drift deposition, runoff generation and filtering, and PECsoil estimation. The approach is spatiotemporally explicit and operates at scales ranging from local edge-of-field to large landscapes. The outcome can be aggregated and presented as comprehensible endpoints to the risk assessor, considering ecologically relevant dimensions and scales for species protection. The approach also assesses the effect of mitigation options, e.g., field margins, in-field buffers, or drift-reducing technology. Presented scenarios start with a schematic edge-of-field situation and extend to real-world landscapes of up to 5 km x 5 km. A case study was conducted for two active substances of different environmental fate characteristics. Results are presented as a collection of percentiles over time and space, as contour plots and as maps. The results show that exposure patterns of off-field soil organisms are of a complex nature due to spatial and temporal variabilities combined with landscape structure and event-based processes. Our concepts and preliminary analysis demonstrate that more realistic exposure data can be meaningfully consolidated to serve in standard-tier risk assessments. The real-world landscape-scale scenarios indicate risk hot-spots which support the identification of efficient risk mitigation. As a next step, the spatiotemporally explicit exposure data can be directly coupled to ecological effect models (e.g., for earthworms or collembola) to conduct risk assessments at biological entity levels.

#### **4.07 Legacy of War: Environmental Contamination, Ecotoxicity and Human Health Concerns of Explosives and Chemical Warfare Agents**

##### **4.07.T-01 Disposal of ammunition from World War One (1914 -1918) on the western front by Open-Burning. The fingerprint of 100-year old forgotten contaminations**

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During the World War One, ammunition was used on an unprecedented scale. When hostilities ceased, huge ammunition dumps remained as fired unexploded ordnances. There was an urgent need to dispose of 2.5 to 3 million tons of hazardous ammunition during the interwar period. More than 200 old ammunition destruction sites have been identified in France. New methods for safely breaking down projectiles, including Open-Burning (OB), were subsequently developed and operated, causing severe contaminations of topsoil and likely contributing to the contamination of groundwater by perchlorate and nitroaromatics compounds (NAC) on a regional scale. However, few studies (often fragmentary) are available on this topic. Recent research was carried out on 9 abandoned burning-grounds in France and Belgium to fingerprint the contamination and assess risks to land and groundwater use. Because the possible presence of warfare material and extremely high concentrations of hazardous compounds, special methods have been developed to safely sample OB residues and soils. Metals, NAC, chemical warfare agents and their by-products, chlorinated and brominated dioxins (PCDD/F, PBDD/F) were analyzed. Parts of munitions have also been collected to be characterized by EOD experts. No vegetation grows at some OB locations due to extreme contamination by heavy metals (Zn: 10 to 100 g/kg DM, As: 2 to 110 g/kg DM) and PCDD/F (1,000 to 4,000,000 ng/kg DM). Isomer distribution patterns indicate that PCDF were the preferred products of burning when primary precursors are chlorinated diphenylarsenical compounds, while  $\sum$ PCDF equal  $\sum$ PCDD when monoaromatic compounds and/or sulfur mustard (dichlorodiethyl sulfide) was decomposed. There are some historical and geoscientific evidences that the fires were out of control with temperature ranging from ca. 300 °C to up to 1,500 °C, over PCDD/F pyrolysis temperature. PCDD/F formation in OB fires may drastically increase when temperature decreased in the presence of precursors mass excess. Inorganic arsenical compounds were associated with organic As by-products where arsenical shells loaded with the sternutator diphenylchlorarsine were open-burned. The fingerprint of contamination is to be linked with the composition of the ammunition being destroyed, and the manner to operated burnings, giving i) valuable historical information that is missing in archive records, and ii) data how to assess OB sites in order to protect public health and environment.

#### **4.07.T-02 Monitoring environmental contamination from relic munitions in the Baltic Sea using lab-based methods and novel fieldable instrumentation**

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The environmental contamination from relic munitions (i.e., the explosive chemicals TNT, RDX, and DNB) has been mapped throughout German waters of the Baltic Sea on a near-annual basis since 2018. This monitoring relies on highly sensitive and selective methods using solid-phase preconcentration, liquid chromatography, and high-resolution mass spectrometry. Observed patterns show clear hotspots of contaminant release from particular munitions dumpsites, which has been confirmed by visual verification of exposed explosives at these sites. Spatial distributions indicate that the type of chemical contamination varies among dumpsites, likely due to sorting of munition types during demilitarization dumping activities. Interannual variability evident for e.g., TNT at the major dumpsite Kolberger Heide, suggests that chemical release is not constant over time, and that regular monitoring is necessary to evaluate environmental risk. However, lab-based methods are extremely time- and effort-intensive, making such monitoring challenging. A new lab-in-a-box system, the Xplotector, has been developed for automated, shipboard analysis of munition chemicals at the sub-ng/L level in seawater. The southwest Baltic Sea and Danish straits were successfully mapped in October 2023, and showed TNT levels generally less than 1 ng/L. Low TNT concentrations were also detected directly above piles of apparently intact munition objects, whereas concentrations increased by many orders of magnitude near corroded munitions. These real-time analysis capabilities allow responsive sampling during monitoring, and greatly speed data acquisition for mapping. Advances in automated analysis of organic contaminants have the potential to revolutionize chemical monitoring for example during munitions clearance or extreme weather events.

#### **4.07.T-03 Life-History Effects of Munition-Related Chemicals in the Copepod *Nitocra spinipes*: Single Chemicals and a Mixture**

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The extensive disposal of munition, including conventional explosives and chemical warfare agents, in global coastal and off-shore sites post-World Wars I and II has led to concerns about environmental contamination. Due to decades of exposure to seawater, munition shells are anticipated to be highly corroded, releasing toxic chemicals into the environment. *Nitocra spinipes*, a copepod benthic species inhabiting these coastal areas, is crucial for connecting microphytobenthos to higher trophic levels in marine systems. Despite escalating environmental concerns, the toxic potential of dumped munition and their degradation products remains largely unknown.

This study provides insight into the impact of seven munition-related chemicals, i.e. thiodiglycol, 1,4-dithiane, 1,4-oxathiane, TNT, tetryl, 1,3-DNB and picric acid, and a mixture on the life cycle of *Nitocra spinipes*, while mimicking as closely as possible the North Sea environmental conditions. Internationally accepted guidelines were used to assess the impact on larval development, focusing on pre-adult mortality and larval development ratio (ISO 18220), and reproductive endpoints, namely inter-brood time and brood size (ENV/JM/MONO(2014)17). Despite conservatively chosen concentration ranges, all munition-related chemicals significantly impacted larval development endpoints. Notably, 1,4-dithiane affected developmental endpoints yet at environmentally unrealistic concentrations, while tetryl emerged as the most toxic E&RC, showing significant effects at environmentally relevant concentrations. The chemical mixture led to severe pre-adult mortality at lower concentrations (in the range of low µg/L) than individual chemicals, indicating synergistic interactions. Reproduction test results generally exhibited non-monotonic concentration-response patterns, with large intra-concentration variability. Exceptionally, tetryl caused significant delays in inter-brood time, and picric acid reduced brood size, suggesting particular concern in scenarios of chronic exposure.

The study demonstrates the effects of the tested munition-related chemicals on two key indicators of population ecology, with potential implications for the marine food chain. The high pre-adult mortality observed from exposure to the chemical mixture and the reported sub-lethal effects underscore the need for more chronic studies involving different species to comprehensively understand the impact of munition dumpsites on marine ecosystems.

#### **4.07.T-04 How Contaminated is our Fish with Munition Chemicals from World War Relics**

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Seas worldwide are threatened by an emerging source of pollution: millions of tons of all kinds of warfare materials were dumped after World Wars I and II. The global amount of unexploded ordnances (UXO) is hard to quantify. However, after more than 70 years the metal shells of these munitions are corroding, such that energetic compounds (EC) leak out, distribute in the marine environment and contaminate surrounding sediment and water. In addition, EC are absorbed by aquatic organisms and pose a threat to both the marine ecosphere and human seafood consumers.

EC like TNT are known for their toxicity and potential carcinogenicity. Therefore, persistent contamination of the marine ecosystem may cause adverse effects to marine life, and directly affect human health via entry into the marine food chain. To date, little is known about the bioavailability, accumulation, distribution, metabolism, detoxification, excretion, and *in vivo* toxic properties of EC from sea-dumped munitions.

In the present study, sediment and fish were examined in defined areas of the North Sea (Lower Saxony) to determine whether EC transfer to fish living in these regions. Of special interest was to infer if EC are present in the edible part (fillet) of the fish and if this endangers the human seafood consumer. Sediment and fish (*Limanda limanda*; dab) samples were collected, processed and analyzed for the presence of different EC by gas and liquid chromatography coupled with mass spectrometry.

In the sediments EC were found in trace concentrations of approx. 1 ng/kg dried sediment. Next, a total of 33 fillet and bile samples were examined for different EC. All fish bile samples were positive for EC detection. Mean concentrations ranged below the one-digit ng/ml for TNT and its metabolites. EC residues could also be measured in the fillet of the flatfish. While TNT metabolites were below 1 ng/g of dry weight, TNT itself was found in concentrations of around 2 to 4 ng/g dry weight. This is interesting, because, as was the case for fish bile, higher concentrations of the TNT metabolites were to be expected in the fish fillet, rather than parent TNT. Therefore, we hypothesize an absorption of EC into fish fillet other than per diet.

We conclude 1) EC from corroding World War munitions transfer into marine biota; 2) EC appear in the fish fillet and might therefore endanger human seafood consumers; 3) TNT obviously enters fish through an additional route, e.g. by gills, in addition to diet.

#### **4.07.P Legacy of War: Environmental Contamination, Ecotoxicity and Human Health Concerns of Explosives and Chemical Warfare Agents**

##### **4.07.P-Tu386 Munitions at Sea: Making ecotoxicological sense out of a littered seafloor – spatial and temporal considerations**

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Underwater unexploded ordnance and discarded military conventional munitions, collectively referred to as underwater military munitions (UWMM), present challenges due to both explosive blast (safety) considerations and potential ecological and human health impacts resulting from the release of munitions constituents (MC) to the aquatic environment. UWMM have the potential to corrode, breach, and leak MC into aquatic environments. When undissolved MCs still contained within a munition enters the aquatic environment, they are not immediately released; environmental releases only occur after the munition is breached by corrosion or mechanical breakage. Breaching can occur either through explosion, mechanical breakup on impact or through corrosion. Historically, large quantities of munitions have been disposed of in the oceans and in large lakes. Despite the large mass of MC present within UWMM at some sites, exposure measurements and estimates have shown that MC is typically present below the limit of detection or at low concentrations (e.g., single digit µg/L). Site characterization efforts at coastal sites have revealed relatively small areas with high concentrations associated with point sources of release with the contamination decreasing orders of magnitude at short distances (e.g., 1 m). As a result of the heterogeneous distribution of contamination, the majority of the organisms living at UWMM sites are likely unaffected by MC present in the water column and sediment, but sessile or low motility organisms, including corals and larval fish, living in those areas of localized higher MC concentrations may be adversely impacted. Potential ecological risk to biota at well-investigated UWMM sites, as well as approaches for site characterization and risk management for UWMM sites will be discussed.

##### **4.07.P-Tu387 Depuration Kinetics of TNT and its Metabolites in lab Exposed Blue Mussels (*Mytilus edulis*, L.)**

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Explosive chemicals released by dumped warfare material pose a threat to the marine environment and can enter the marine food web. These chemicals are toxic and are suspected to be carcinogenic, mutagenic and have also genotoxic effects. 2,4,6-Trinitrotoluene (TNT) is one of the most used explosives in munitions and therefore of special interest. To test the potential uptake, bio-concentration or bio-accumulation, and metabolism or depuration of TNT in marine organisms two lab exposure experiments were conducted using common blue mussels (*Mytilus edulis*). Mussels were exposed to different TNT concentrations for a specific period of time. The first experiment ran for a total of 120 hours, with 48 hours of exposure to different TNT concentrations (0 mg/L, 0.625 mg/L, 1.25 mg/L and 2.5 mg/L) followed by a 72 hours recovery phase in clean artificial seawater. Mussel sampling took place during the recovery phase.

The second experiment was performed as follow up to the first experiment and was conducted over 36 hours, with 24 hours of exposure and 12 hours of recovery. Water and mussel samples were taken during exposure and recovery phase with a higher sampling resolution within the first hours of the recovery phase.

GC-MS/MS was used to determine the mussel tissue and water concentration of TNT and its metabolites 2-amino-4,6-dinitrotoluol (2-ADNT), 4-amino-2,6-dinitrotoluol (4-ADNT) and 2,4-diamino-6-nitrotoluene (2,4-DANT).

Results of the first experiment showed a rapid decrease in TNT and metabolite concentrations in mussel tissues over time. Overall, 57 to 76 % of the detected concentration was metabolised within the first four hours after the exposure. Analysis of samples of the second experiment verified the results from the first experiment showing clear linear digression of the two ADNTs within the first four hours of the recovery phase and a more or less complete metabolism of the parent compound TNT.

In summary, uptake of TNT in relation to the exposure concentration could be proven and first insights concerning the metabolism and depuration velocity could be shown.

#### **4.07.P-Tu388 Screening Concentrations for Ecotoxicological Assessment of Soils Contaminated with Explosives Residues**

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Many sites associated with ongoing operations in Eastern Europe and the Middle East, as well as those that involve munition manufacturing, disposal, testing, and training contain elevated levels of explosives, propellants, and related energetic materials (EM) in soil. We conducted definitive toxicity studies with terrestrial plants and soil invertebrates to establish receptor-specific toxicity benchmarks for nitrogen-based organic energetics RDX (hexahydro-1,3,5-trinitro-1,3,5,-triazine), HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine), TNT (2,4,6-trinitrotoluene), TNB (1,3,5-trinitrobenzene), 2,4-DNT (2,4-dinitrotoluene), NG (1,2,3-trinitroglycerine), DNAN (2,4-dinitroanisole), and NTO (3-nitro-1,2,4-triazol-5-one), each weathered-and-aged up to three months in Sassafra or Teller sandy loam soils. Toxicity benchmarks were derived using the EC20 level of the EM effects on plant growth or soil invertebrate reproduction measurement endpoints determined from standardized toxicity tests. Soil screening concentrations for an EM-receptor pairing were calculated as the geometric mean of EC20 toxicity benchmarks determined from the individual studies. Toxicity benchmarks determined in these studies have filled the data gaps in current knowledge of the potential ecological risks of release of EM compounds into soil and can be used to develop risk-based approaches to assessing exposure risks and developing remedial goals for contaminated soils.

#### **4.07.P-Tu389 Calculation of the dynamics of special unexploded ordnance (UXO) for rapid recovery and avoidance of toxic environmental contamination**

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Unexploded Ordnance Devices (UXO) and Discarded Military Munitions (DMMs) pose a persistent and alarming threat in coastal regions, primarily due to their exposure to environmental forces like saltwater corrosion, wave dynamics, currents, and shifting seabed sediments. This threat extends beyond human safety to encompass the environment, with a specific concern regarding the release of toxic substances.

These remnants of war continue to pose a latent threat to human life, particularly in areas where offshore construction activities occur. Extensive surveys are conducted to locate, identify, and clear these hazardous objects as needed. However, the danger persists even after clearance operations. The possibility of these objects being mobilised by currents or waves, whether in cleared areas or those left undisturbed, remains a critical concern.

To assess the potential for object mobilisation, it is essential to calculate the critical loads required to displace UXO from their stable positions within the sediment bed. These loads depend on factors such as hydrodynamic and hydrostatic forces, friction, gravity, and inertia.

This study employs a comprehensive approach to analyse the underlying forces. Hydrodynamic forces are rigorously examined using a combination of analytical methods and numerical simulations. The determination of the dimensionless coefficients is done by wind tunnel experiments. The study also establishes the complex relationships between the dimensionless coefficients for lift, drag and additional mass, considering the Reynolds number and burial depth.

The study uses model simulations based on fluid mechanics to quantify the forces acting on these objects through the interaction of waves and currents. For this purpose, critical flow velocities and wave conditions required for the mobilisation of various objects are determined and these results are compared with existing environmental conditions, especially in the Fort Pierce region in Florida, USA.

This research and its outcomes provide the means to forecast the underwater path of UXO situated on the seabed. Such predictive capabilities can profoundly reduce the substantial hazards posed to human safety and mitigate the environmental risks associated with the release of toxic substances. Additionally, this information can be leveraged to enhance the efficiency of surveys, thereby expediting offshore construction projects and markedly enhancing the safety of coastal areas.

#### **4.07.P-Tu390 Heritage of war - a multi-biomarker approach to investigate the effects of sunken war wrecks on common dab (*Limanda limanda*) in the North Sea**

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Today, the North Sea is the final resting place for hundreds of shipwrecks from the two world wars. Many of these ships were still partially or fully loaded with munitions at the time of their sinking. The increasing corrosion of munition shells in the saline environment leads to an increased leakage of chemical substances into the marine ecosystem posing risks to marine organisms. A large amount of the chemicals preserved in the munitions are toxic and classified as CMR substances (carcinogenic, mutagenic, toxic for reproduction). Particularly, the 2,4,6 - trinitrotoluene (TNT) is of great concern here, since it has been the most commonly used explosive during the world wars. The accumulation in the tissue of various organisms has been proven in previous studies, but the knowledge about the biological effects of TNT on marine organisms is still limited.

To investigate the biological effects of World War munition on marine organisms, field studies were conducted on three selected wrecks (SMS Ariadne, SMS Mainz, UC30) from the First World War in the southern North Sea, as well as from a suitable reference area. Therefore, non-migratory dabs (*Limanda limanda*) were caught as close as possible to the wreck sites and investigated using a multi-biomarker approach. The dabs were first examined for obvious alterations in the liver before being dissected for subsequent assessment of relevant tissues.

The results show that dabs fished near the wrecks react negatively to the elevated TNT concentration in the water. The percentage of dabs with liver alteration is significantly increased in the fish caught at the wreck sites compared to the dabs from the reference area. Similarly, those results can also be mirrored at the cellular level. Elevated TNT concentrations in the water lead to the accumulation of metabolic end products in the liver tissue and alter the activity of enzymes involved in the anti-ox-defense system.

These results correlate with the TNT concentrations detected in the water and therefore suggest a negative impact of the explosive compounds still left on war wrecks on marine organisms.

#### **4.07.P-Tu391 Bacterial Clues of Shipwreck TNT Pollution in the North Sea**

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Almost 300 shipwrecks are found in the Belgian part of the North Sea. At the time of sinking, many were armed and fully or partially loaded with ammunition. One of the most important substances in this context is 2,4,6-trinitrotoluene (TNT). Commonly used as an explosive for military applications, TNT is equally known for its toxicity as well as its potentially mutagenic and carcinogenic properties. Corrosion ultimately results in the leakage of dissolved explosives from lost munitions in shipwrecks, causing the surrounding sediments to be contaminated by TNT and related organic compounds. Marine biota like fish and shellfish bioaccumulate these compounds, thereby creating a risk to human seafood consumers. By analysing changes in the marine microbial community in the presence of TNT and its metabolites, we aim to identify bacterial taxa that can serve as indicators for pollution of dissolved explosives from shipwrecks.

This study investigates the link between dissolved explosives and the microbial fingerprint in sediments next to a WWII destroyer. This wreck still has semi armour-piercing shells on board, which are leaking low levels of TNT into the surrounding sediments. Samples were taken by divers at 10-meter increments away from the wreck and were analysed for their concentrations of dissolved explosives using gas chromatography combined with mass spectrometry (GC-MS). Next, 16S rRNA gene long-read nanopore sequencing will be explored as a rapid, cheap tool to map the microbial composition of sediment samples. Previous studies show a sensitive shift in microbial fingerprints in the presence of aromatic pollutants. Here, we expect to find changes in the microbial community, potentially towards TNT-degrading taxa, in samples with higher concentrations of dissolved explosives.

#### **4.07.P-Tu392 Spatial Distribution of WWII Legacy Munition Compounds in the Baltic Sea.**

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After about eight decades in the sea, WWII legacy dumped munitions are at different stages of corrosion and continue to leak chemicals into the water column and sediment. Rise in demand for renewable energy, conservation of biodiversity and reduced risk of human exposure to munition compounds (MCs), to mention but a few, necessitate assessment of the current status of dumpsites, exploration of cleanup options and prioritization of sites for cleanup. To this end, mapping of the MC pollution in the Baltic Sea, particularly in the German Waters, is ongoing. Water and sediment samples are collected and analysed for MCs using an Ultra High-Performance Liquid Chromatograph coupled to a High-Resolution Mass Spectrometer. Water samples collected in October 2022 show the major hotspots of current TNT release to be in the Kolberger Heide – Falshöft sections (reaching 10 ng L<sup>-1</sup>) whereas the Lübeck Bay is a major hot spot for DNB and RDX emissions (reaching 75 ng L<sup>-1</sup> at a site off Haffkrug and 37 ng L<sup>-1</sup> at a site off Neustadt, respectively). Transformation products of TNT, 2-ADNT and 4-ADNT were detected in all water samples, the highest concentrations being observed at Kolberger Heide (1.4 and 3.4 ng L<sup>-1</sup>,



respectively). A more detailed discussion of the spatial distribution of key MCs and transformation products of TNT measured in the entire stretch of the German waters, and along the Little Belt – Aarhus –Great Belt - Femer Belt section of the Danish waters of the Baltic Sea, will be presented.

#### **4.07.P-Tu393 Biological effects of munition left on war wrecks on the health of blue mussels (*Mytilus edulis*, L.) in the North Sea**

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The North Sea was the scene of many naval battles during both World Wars in which a large number of military ships, civilian merchants, and cargo vessels sank. Many of those ships were still partially or fully loaded with munition at the time of their sinking. However, only rough estimates about the remaining quantities of munition on these wrecks can be made. After decades in the marine environment, munition shells are corroding and start leaking chemicals of their explosive cargo into the marine environment. As the main component of most munitions, 2,4,6 trinitrotoluene (TNT) is of great importance here. Next to its ability to explode, TNT is also known for its toxicity and for being carcinogenic and mutagenic.

To test for leaked explosives in the surrounding waters, sediments and biota field studies were conducted on three wreck sites in the North Sea. Water analysis shows different TNT concentrations at the wrecks. The measured concentration of explosives is subsequently correlated with potential health impairments in exposed blue mussels. These mussels were exposed for several weeks in steel cages on or near the three wreck sites. Three to four cages with blue mussels were placed at different locations on each of the wrecks. At the end of the exposure time mussels were retrieved and examined for mortality, subsequently dissected and analyzed in the lab for biomarker responses according to a multi-biomarker approach.

The first results show differing water concentrations of dissolved TNT around the wreck sites ranging from low ng/L of dissolved TNT at SMS Mainz up to the µg/L and mg/L levels at UC30 and KW58. Furthermore, they show that higher TNT concentrations at the wrecks trigger biological responses in the mussels. Elevated TNT concentrations lead to increased activities of enzymes involved in the anti-ox-defense system and to higher accumulations of metabolic end-products in cells of the digestive tissues of the mussels, as a response to the presence of organic pollutants, suggesting a negative impact of TNT dissolved in the water on the exposed mussels.

#### **4.07.P-Tu394 Ecotoxicological risk of World War relic munitions in the sea after low and high order blast in place operations**

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Submerged munitions from both World Wars (WW) are present in marine waters across the globe and are threatening human activities in the oceans, including fisheries and shipping or the construction of pipelines and offshore facilities. Several studies have shown that TNT and its metabolites are carcinogenic and exhibit acute and chronic toxic effects in a wide range of marine organisms.

Remotely controlled high-order blast-in-place (BiP) operations are used by navies worldwide to clear underwater munitions. This involves detonating the explosives in the mine with an external explosive charge. The resulting shock waves pose a serious threat to marine mammals such as harbor seals and harbor porpoises as high sound pressure and explosion-related shock waves can lead to severe injuries and hearing impairment in marine mammals in great distances from the detonation site. Low-order BiP operations are being discussed as a marine mammal-friendly alternative. With this form of remediation, not all of the explosive material is detonated, which minimizes the shock wave.

Previous studies have proven that after underwater high-order BiP detonations, toxic energetic compounds (EC) such as TNT will not completely combust, but rather distribute within the marine ecosphere. It is therefore to be expected that even larger quantities of EC will be released during low-order BiP operations.

Two British MK I-IV ground mines were chosen for detonation using high-order (Mine Mike) and low-order (Mine Golf) BiP operations. Water and sediment samples were taken at 0, 0.5 and 1 m in all four directions prior to and in 0, 1m and 5 m in the right direction immediately after the respective BiP operation by Danish Royal Navy divers. Samples were extracted in the lab and analysed by sensitive GC-MS/MS and LC-MS/MS technology for their concentrations of the EC 1,3-DNB, 2,4-DNT, TNT, and its metabolites 2- and 4-ADNT.

Our study provides unequivocal evidences that BiP operations in general lead to a significant increase of contamination of the marine environment and ecotoxicological risk with toxic EC. Although the noise emission and shock wave are considerably lower with low-order BiP operations, they result in a several thousand-fold higher burden on the marine environment. For this reason, we explicitly criticize both types of BiP operations because of the resulting environmental hazards, which can ultimately even endanger human seafood consumers.

#### **4.07.P-Tu395 Demonstration of a Commercially Available Passive Sampler for Monitoring of Munitions Constituents at an Underwater Naval Training Range**

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As a result of military activities, discarded military munitions and unexploded ordnance are present in underwater environments, which has resulted in the release of munitions constituents (MC) including the high explosives 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), to the water column and adjacent sediments. This paper focuses on the environmental characterization of exposure (i.e. aqueous concentrations) of these constituents in an underwater embayment having relatively high densities of underwater unexploded ordnance (UXO), specifically the Vieques Naval Training Range at Bahia Salina del Sur (Vieques, Puerto Rico, USA). Following multiple calibration efforts, in situ passive sampling using polar organic chemical integrative samplers (POCIS) was employed for the detection and quantification of MC in water adjacent to potentially leaking munitions. Laboratory calibration and field demonstration of HLB POCIS (alongside grab sampling) verified that passive sampling was a useful tool towards the estimation of time averaged water concentrations for MC including TNT, RDX, and their primary degradation products at the site. POCIS-derived and averaged grab water samples agreed within a factor of 3. When detected, MC concentrations were observed at ultra-trace concentrations (as low as 4 ng/L for RDX), except 30 cm from one General Purpose bomb where the TNT concentration was 5.3 µg/L, indicating that low level contamination existed at the site on a localized scale despite the relatively high density of munitions, similar to previously reported results for other munitions sites around the world. More specifically, POCIS was able to detect MC at levels substantially lower than achievable using typical grab sampling methods, allowing for improved characterization of MC in comparison to grab sampling at underwater sites where very low concentrations (ng/L) or fluctuation in concentrations are expected to occur.

#### **4.08.A Marine and Coastal Pollution: Detection, Monitoring, Assessment, Regulation, and Management**

##### **4.08.A.T-01 Evolving Chemical Assessment for Use in the Marine Environment**

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##### **Introduction**

The aquatic environment is subject to multiple chemical inputs. Chemicals are discharged in offshore oil and gas exploration, many are also used in offshore renewables and form shipping, riverine inputs and aquaculture chemicals. Chemical regulation varies between different sectors and geographic regions and present a significant regulatory challenge and pose difficult questions. For example, how do we control the use and discharge of chemicals and what sort of methods can we use to model risk and fate? Are there any data gaps? Where are the highest areas of concern and uncertainty? A major source of uncertainty is the hazard assessment of unknown variable chemicals or biological substances (UVCBs) which are ubiquitous in our daily lives, in materials and chemicals that are used and discharged. Questions have been raised regarding the suitability of test protocols to help identify environmental hazard concerning UVCBs and reaction mixtures, and whether assessment factors could be applied to reduce uncertainty, especially with mixtures. Many chemicals appear to be readily biodegradable but many are not. While increasing the handling and solubility they also enhance biodegradation by masking persistence. In addition, once any solvent enters the aquatic environment it is diluted and dispersed with smaller more mobile molecules being stripped out of the mixture leaving amorphous solids such as gels and polymer dispersions. There are rightly concerns regarding plastic pollution but on contrast there is less concern raised about other polymers which could lead to the build up of residues in the environment.

Discussion: One way of taking into account masking of persistence is the use of solvent correction calculations. This has been demonstrated on a simple rhodamine B dye and acetic acid as a solvent and could apply to mixtures where excess chemicals are present. There are additional issues that need to be addressed, e.g. whether mixture effects are important and whether assessment factors can be applied to identify safe use of products or substances, and whether this could be applied to UVCBs and reaction mixtures where the toxicity of individual components may be known? How can we obtain chronic information without increasing animal testing? There are guidelines available that can help address the need for chronic data, but further work is needed for regulators to understand the New Approach Methodologies (NAMs) endpoints to be able to apply them.

##### **4.08.A.T-02 Chemicals in OWF Coatings and their Leaching into the Marine Environment**

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Turbine coatings used for corrosion protection on offshore wind farms (OWFs) are in direct contact with the surrounding seawater, while leading edge erosion of the rotor blades can result in the formation and release of coating particles to the marine environment. Both of these exposure routes present an opportunity for additive chemicals and non-intentionally added substances (NIAS) to leach from the coating materials into the seawater, where they can be bioavailable to marine organisms.

In the current study, chemicals reported used in coatings were reviewed and a standard operating procedure for laboratory investigation of chemicals leaching from paints and coatings commonly used at North Sea OWFs into seawater was developed and applied.

Data from the literature review was compiled, listing known chemicals, as well as available chemical and hazard information, and classifying all chemicals according to the emission source/s. The primary focus was on organic substances potentially emitted by coatings, but the list also includes chemicals released from other sources within OWFs like sacrificial, firefighting foams or operational fluids. Different groups of chemicals (e.g. phenolic compounds, diisocyanates, heavy metals, poly- and perfluorinated compounds) were identified and prioritized for further analysis.

To investigate the leaching of chemicals from OWF coatings, stainless steel coupons sandblasted and painted via air spray using the two most applied commercial paints, with different combinations of layers, primers and top coats. The coupons were subjected to a 7-day leaching study in seawater under low turbulence conditions at 12 °C. Leachate extracts were analysed using a non-target screening method using two-dimensional gas chromatography coupled with mass spectrometry to tentatively identify the chemicals leaching.

The results indicated some matches between reported chemicals in the literature and those chemicals found in experimental seawater leachates. Some of the chemicals expected to be in the coatings were not observed in leachates, possibly because they are not readily water-soluble in the conditions tested (and therefore not expected to be bioavailable in the marine environment) or because they were not amenable for GC-MS analysis. Further work still needs to address the sources of chemicals found in leachates that are ranked as potentially hazardous, but which are currently unreported and thus not subject to environmental risk assessments.

#### **4.08.A.T-03 The Underestimated Problem with Discharge Water from Exhaust Gas Cleaning Systems of Seagoing Ships**

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The introduction of the global limit of 0.50% (m/m) of sulphur emission in 2020, accelerated the number of exhaust gas cleaning systems (EGCS or scrubbers) installed on seagoing ships. Whereas the sulphur bill tackles airborne SO<sub>x</sub> emissions, the operation of scrubbers produces highly toxic wash water, which is released into the marine environment. The present project aimed to assess whether the current discharge criteria of the “2021 EGCS Guidelines” (IMO resolution MEPC.340(77)) sufficiently safeguard the protection of the marine environment, based on an ecotoxicological evaluation. Thus, a sampling campaign was conducted on four ships with scrubber hybrid systems and the water samples then chemically and ecotoxicologically analysed. The EGCSs were sampled at several stages of the scrubbing process under normal operating conditions in both operation modes, open-loop (OL) and closed-loop (CL). For the ecotoxicological assessment, a combination of whole effluent toxicity (WET) tests on three marine species (*Aliivibrio fischeri*, *Phaeodactylum tricornutum*, *Acartia tonsa*) and two specific tests for mutagenic (Ames fluctuation test) and dioxin-like effects (YDS - recombinant yeast dioxin screen) was applied. Additionally, fish embryo acute toxicity tests (FET/OECD 236) were included as a non-animal test with a vertebrate species. The WET results showed much higher toxicity of the CL discharge samples (all categorised extremely toxic) than of the OL discharge samples (practically non-toxic to considerably toxic). The highest sensitivity was shown by the copepods, followed by the marine algae. The dioxin-like effects in the YDS were also higher for the CL than the OL discharge samples. The result pattern of the CL samples showed some coherence with the oil content and the 16 EPA PAH concentrations. The Ames tests with the strains TA98, TA100, YG1041 and YG1042, with and without metabolic activation, showed mutagenic potency in all discharge samples. However, OL discharge samples were less mutagenic than CL discharge samples. The undiluted CL discharge water samples of the ships S2 and S4 also caused over 50 % mortality (48 h) and reduced hatching rates of 385 % in the FET. Given these ecotoxicity results, we consider the release of scrubber discharge water from both operation modes into the sea to be of high concern. The effluent is acidic and contains persistent, bioaccumulative and toxic pollutants (PAHs, heavy metals, etc.) which may cause long-term effects.

#### **4.08.A.T-04 Sulphate sensitivity of aquatic organisms in northern Baltic Sea coastal brackish water explored by toxicity tests and species sensitivity distribution**

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Sulphate is a naturally occurring major ion ubiquitously found in natural environments. Major anthropogenic sources of sulphate include runoff from agricultural lands on acid sulphate soils, effluents from industrial activities, as well as mining and municipal wastewaters. Chronic sublethal effects of sulphate on freshwater aquatic organisms have been explored and observed in many toxicity tests, and the development of sulphate environmental quality standard in freshwater has gained more attention recently. However, the effects of sulphate on the brackish water biota are poorly known and experimental data from

biotests are largely missing. We conducted chronic sulphate toxicity tests on species from northern coastal Baltic Sea areas and used Species Sensitivity Distribution (SSD) modeling to derive the 5th percentile hazardous concentration (HC5) that would protect 95% of the species in the brackish water ecosystem where sulphate concentrations range between 100 to 500 mg/L. Chronic sulphate toxicity varied notably between the species tested. The most sensitive species tested were whitefish *Coregonus lavaretus* and common water moss *Fontinalis antipyretica*. This study provides important preliminary understanding on sulphate sensitivity in brackish water and more toxicity tests are needed to include possibly sensitive species not included in this study.

#### **4.08.A.T-05 Toxicity Variations in Produced Water: A Study on Danish Offshore Oil Fields**

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In the oil and gas sector, including carbon capture and storage, produced water (PW) is a significant byproduct. Denmark's offshore oil production annually discharges about 30 million cubic meters of PW, a complex mixture of hydrocarbons, suspended solids, metals, and production chemicals, into the sea. Current regulations, mainly focusing on oil-in-water content, do not fully address the variability in PW's composition, which can harm marine life. The challenge in regulating PW lies in its diverse constituents and the reliance on limited or unclear data for environmental assessments.

This study designed a sampling protocol for offshore personnel to evaluate the toxicity variations in PW from different Danish North Sea oilfields. The research involved 48-hour acute toxicity tests on the copepod *Tisbe battagliai* and *Crassostrea gigas* (oyster) embryos using various dilutions of PW samples. The study examined the toxicity effects of suspended particulate matter (SPM), bivalent cations, and volatile compounds employing filtration, EDTA chelation, and nitrogen wash as intermediate treatments, respectively.

Results showed significant variability in PW toxicity across different fields and sampling times, indicating challenges in developing effective regulations. Factors like SPM, volatiles, and toxic elements (Cu and Zn) notably influenced organisms' responses, with variations even within samples from the same field.

In conclusion, the study emphasizes the complexity of regulating PW due to its variable toxicity. As the oil and gas industry evolves, monitoring PW's composition and eco-toxic effects becomes crucial. The Danish Underground Consortium aims for "zero harmful discharge" by 2050, highlighting the need for comprehensive PW monitoring and regulation in subsurface operations.

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#### **4.08.B Marine and Coastal Pollution: Detection, Monitoring, Assessment, Regulation, and Management**

##### **4.08.B.T-01 Impacts of the UV Filters Octinoxate and Octocrylene on *Symbiodinium* sp.: A Symbiotic Microalgae in Corals**

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The widespread use of ultraviolet (UV) filters in sunscreens and personal care products has raised concerns due to their adverse effects on aquatic organisms. This study investigated the specific toxicity of two UV filters, octinoxate, and octocrylene, known for their potential harm to coral reefs. The focus is on *Symbiodinium* sp., a photosynthetic dinoflagellate crucial for the symbiotic relationship with corals, contributing to nutrient acquisition and coral reef structure.

Exposure of exponentially growing *Symbiodinium* sp. to octinoxate and octocrylene revealed significant toxicity. Octinoxate proved three orders of magnitude more toxic than octocrylene, emphasizing the differential impact of these UV filters. The study utilized a comprehensive set of sub-lethal endpoints analyzed through flow cytometry, including cell viability, pigments content, cell size, complexity, metabolic activity, reactive oxygen species production, and membrane potentials.

For octocrylene, sub-lethal effects included increased cell complexity, lipid peroxidation, and altered membrane potential. At higher concentrations, decreased cell viability and metabolic activity indicated severe membrane depolarization. Octinoxate, even at lower concentrations, affected cell size, complexity, viability, and metabolic activity, with an increase in lipid peroxidation levels. The results underscore the potential for structural changes in cellular components.

The study's findings emphasize the importance of an early warning system to protect *Symbiodinium* sp. within coral reef ecosystems. The differential toxicity of UV filters warrants careful consideration in formulating effective conservation strategies. Flow cytometry emerges as a valuable diagnostic tool for detecting sub-lethal effects, providing insights into the

health of *Symbiodinium* sp. and, by extension, the resilience of coral reef ecosystems. Overall, this study contributes valuable insights into the specific impacts of UV filters on a key component of coral reef ecosystems and advocates for proactive measures to safeguard these vital environments.

#### **4.08.B.T-02 Effect-based Water Quality Diagnosis to Improve Coral Reef Resilience and Recovery**

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In the marine environment, and in particular on coral reefs, the presence of complex mixtures of pollutants and especially their effects are still poorly understood. Coral reefs are exposed to low-level and at times highly variable toxic pollutant mixtures, and passive sampling is particularly fit for these conditions as it allows for the *in-situ* concentration of analytes and the integration of extended periods. Target chemical analyses overlook unknown chemicals and are unable to integrate the toxic effects of complex mixtures. Effect-based methods (EBM) can overcome these limitations by providing a risk-scaled assessment of the mixture toxicity elicited by all known and unknown bioactive chemicals in a sample. Hence, the combination of passive sampling and EBM is particularly fit to monitor the complex low-level mixtures of chemicals that can threaten the health of coral reefs and help prioritize sites that are suitable for coral conservation and restoration efforts. However, although this has been developed for freshwater systems, an approach to monitoring chemical pollution in coral reef ecosystems using passive sampling and EBM is currently not available. Here, we present the results of a pilot and follow-up study, in which polar organic chemical integrative samplers (POCIS) were exposed at multiple inland bays and coral reef sites on the island of Curaçao in the Southern Caribbean. POCIS extracts were exposed to a battery of bioassays to determine the toxic pressure at the locations, and bioassay responses were compared to effect-based trigger values to identify potential ecotoxicological risks. Extracts from the bays consistently elicited higher bioassay responses than those from their adjacent reef sites, confirming the expected dilution of pollution as it is transported from land onto the reefs. This was also reflected in the cumulative ecotoxicological risk quotients for each of the locations. Chemical target and non-target analyses of the POCIS extracts were included in the follow-up study to provide insight into the link between toxicity and the occurrence of pollutants and their sources. Effect-based water quality assessment shows great promise for the identification of local chemical stressors that threaten the health of coral reefs. Further development of these methods is needed to inform local stakeholders and help them control local stressors to improve the resilience and recovery of these valuable ecosystems.

#### **4.08.B.T-03 Mapping Coastal Chemical Contamination in Different Environmental Matrices Across the Galapagos Archipelago**

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As a developing island province, the Galapagos Archipelago is at the forefront of the Anthropocene footprint, with its population influx, urbanisation, reliance on fossil fuels, growing tourism, fishing and agricultural industries cumulatively contributing to increasing pollutant input at the coastal interface. Subsequently, despite its geographical isolation ~1000 km off the coast off South America in the eastern Pacific Ocean, the Galapagos Marine Reserve (133,000 km<sup>2</sup> encompassing the islands) is at risk from oil, plastic, pesticides, persistent organic pollutants and heavy metal contamination; such contaminants threaten to overwhelm the adaptive capacity and resilience of these fragile island ecosystems and the unique biodiversity they support. Here we characterise and quantify coastal chemical contamination in different environmental matrices at the archipelago scale. Surface seawater and benthic sediment samples were collected in triplicate using grab techniques from eleven coastal sites around the three main inhabited Galapagos Islands (San Cristobal, Santa Cruz and Isabella). Novel passive samplers (Markes 'HiSorb' probes) – steel probes coated with PDMS solid phase – were also deployed at each site for a period of 1–3 weeks to further understand the temporal variability of contamination and evaluate their potential use as rapid assessment monitoring tools. HPLC-MS analysis revealed carbendazim and diuron to be the most frequently detected pesticides and found at the highest concentrations (43.75% and 37.5% of samples; 24.6 ng/L and 24.9 ng/L, respectively). Proximity to urbanisation and point-sources appeared to influence levels of pesticide contamination, with enclosed brackish waterbodies exhibiting the highest concentrations. Initial forensic GC-MS screening of compounds bound to HiSorb probes has identified medium-to-long chain hydrocarbons, chemical ingredients associated with sunscreen products and residues of the widely used insect repellent DEET. This investigation provides valuable baseline data on chemical contamination across the Galapagos Archipelago to inform future management and mitigation strategies by the Galapagos National Park. Over the next decade, a complex trade-off between urbanisation and conservation must be carefully navigated to ensure the preservation of this UNESCO World Heritage Site in this evolving chemical landscape.

#### **4.08.B.T-04 Microplastics as Toxicant Vectors: Comparisons with Natural Particles in a Marine Trophic Web**

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Microplastics (MPs, 1 µm - 5 mm) are a major aquatic environmental issue, linked to various effects on marine organisms. MPs pose risks beyond direct impacts, as they can transport environmental pollutants. While trophic transfer of MPs has been demonstrated, the fate of pollutant-loaded MPs in the food chain remains underexplored. Additionally, organic particles present in marine environments like microalgae (MA) can also act as pollutant carriers, making it crucial to compare the roles of MPs and MA in transporting highly bioaccumulative and toxic pollutants, such as mercury (Hg). Therefore, a three-level

model trophic chain based on phytoplankton (*Rhodomonas lens*), copepods (*Acartia tonsa*) and fish (*Scophthalmus maximus*), was chosen to study the individual and combined effects of Hg, high-density polyethylene MPs and MA in *S. maximus* larvae (16-31 days post-hatching). Over 15 days, larvae were fed 1,000 adult *A. tonsa*/day from each condition: (1) Filtered seawater control; (2) 4 µg Hg/L waterborne Hg (WBHg), (3) 3,000 part/mL oxidized MPs (Ox MPs), (4) 3,000 part/mL and 4 µg Hg/L (Ox MPs+Hg), and (5) 3,000 part/mL and 4 µg Hg/L. Hg-loaded *R. lens* (MA+Hg). Each test group had four replicates, each containing 30,000 *R. lens*/mL. No statistically significant differences were observed among experimental conditions in survival rate, total length, Fulton's condition index, and copepod consumption by larvae at the end of the experiment. Larvae exposed to Ox MPs+Hg and WBHg exhibited similar bioaccumulation levels over the exposure period, being significantly different from larvae exposed to MA+Hg and control after 3 and 7 days of exposure. After 7 days, MA+Hg treatment showed significantly higher acetylcholinesterase activity than Ox MPs and WBHg. At day 3, larvae exposed to Ox MPs exhibited higher GST activity than all other conditions except MA+Hg. However, by day 7, Ox MPs showed significantly decreased GST activity compared to control and MA+Hg. No significant differences in GRx activity were observed at 3 and 15 days across conditions, but after 7 days, larvae exposed to MA+Hg exhibited a significant increase compared to Ox MPs and WBHg. MPs transported Hg in the studied trophic chain as larvae exposed to Ox MPs+Hg showed bioaccumulative and toxic effects comparable to WBHg. MA may prevent Hg bioaccumulation, while inducing an adaptive response against oxidative stress. These results emphasize the need for comprehensive environmental health management.

#### 4.08.B.T-05 Antibiotic Occurrence and Distribution in a Coastal Areas with Aquaculture Activity.

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The presence of antibiotics (AB) in the environment can be originated from both human and veterinary sources and can pose a risk to non-target species and to contribute to the spread of antibiotic resistance. AB can permeate surface waters, coastal regions, and groundwater, and can disrupt aquatic ecosystems and eventually impact human health. Aquaculture, a crucial source of fish for human consumption, raises concerns due to how environmental pollution affects this industry. This study aims at investigating antibiotic occurrence in coastal marine environment and in aquaculture facilities located in such areas (mariculture).

Conducted in Porto Venere, Liguria, Italy, at a commercial "antibiotic-free" aquaculture plant, the study encompasses two sampling campaigns in summer 2022 and winter 2023. Sampling sites, selected along a putative pollution gradient, include a negative control sample (CASTAGNA), four fish cages within the aquaculture plant, an intermediate site (PONTILE), and a semi-submerged channel (CANALE) influenced by urban discharges and tidal variations.

Water samples underwent filtration and pre-concentration using solid-phase extraction (Oasis HLB cartridges). Sediments were processed using a QUEChERS methodology and fish fillets and benthic biota were analyzed using zirconium beads beating. A comprehensive range of 27 AB and four human metabolites, classified into 10 distinct chemical groups, were monitored using LC-MS/MS. Environmental risk assessment was conducted in water and sediments by calculating risk quotients (RQ) categorized as no risk ( $RQ < 0.1$ ), moderate risk ( $0.1 < RQ < 1$ ), and high risk ( $RQ > 1$ ).

Results indicate sulfonamides and macrolides as the most abundant AB in mariculture seawater, with higher concentrations in winter. Sediment samples revealed a diverse AB presence, with ofloxacin recording the highest concentration. Fish and biota analysis identified specific AB. Despite the aquaculture farm being antibiotic-free, the presence of AB suggests potential external sources and anthropogenic contamination.

Risk assessment outlines moderate and high risks for certain AB in seawater and sediments, emphasizing potential threats to the marine environment. These findings underscore the need for ongoing monitoring and mitigation efforts to protect marine ecosystems and maintain the integrity of aquaculture practices.

#### 4.08.P Marine and Coastal Pollution: Detection, Monitoring, Assessment, Regulation, and Management

##### 4.08.P-Mo362 Contaminants of Emerging Concern in the Marine Environment: An Integrated Effects Assessment Approach (CONTRAST)

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The Horizon Europe project, CONTRAST that started in January 2024, will develop an integrated assessment and effect-based

monitoring framework (IAF) to measure the impacts of contaminants of emerging concern (CECs) on the marine environment, which will contribute to the assessment of Good Environmental/ Ecological Status for application in EU policy (i.e., MSFD/WFD). The IAF will involve chemical measurements together with biological effects endpoints that will be optimised to detect the presence and degree of effect of CECs in the marine environment. CONTRAST will identify the CECs that pose the greatest threat to marine life by using chemical prioritisation schemes to inform which of the CECs should be measured in the environment. Additionally, which CECs to target in the laboratory experiments, where the effects on biological systems and marine biodiversity will be assessed. A combination of *in silico*, *in vitro* and *in vivo* bioassays together with omics will be used to determine the mechanisms of toxicity of selected CECs. This will provide important information on how CECs may interact with marine organisms at environmentally relevant concentrations and inform which biological effects tools should be used in the IAF to cover the range of toxicity mechanisms that CECs produce. CONTRAST will address this by optimising existing and developing new biomarkers, so that the IAF includes biomarker tools to cover the most relevant mechanism of toxicity produced by CECs. A series of European-wide case studies will be used to test the suitability of the IAF to measure the effects of chemicals including CECs on indicator species and biodiversity. The knowledge gained from field testing and laboratory studies will form the basis for guidance documents and policy briefs on the best practices for performing an IAF on CECs in the marine environment and help to provide the necessary protection of marine ecosystems. Furthermore, the interactions of climate change drivers on CECs will be evaluated including effects on CECs distribution, circulation, fate, bioavailability, and toxicity to marine life. CONTRAST is funded by the European Commission within the Horizon Program.

#### **4.08.P-Mo363 Baltic Sea Biological Effects Activity Cluster: Joint Regional Activities for an Improved Assessment of Chemical Pollution in the Marine Environment**

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Chemical pollution impacts the health of marine biota, key functions of the ecosystem, and biodiversity. The current environmental monitoring and assessment approach is focused on chemical and ecological measurements, while linking biological effects to chemical exposure, has been challenging. Although the contaminant assessment using single compounds is of utmost importance for environmental quality standards, taken alone it does not ensure compliance with Descriptor 8 of the Marine Strategy Framework Directive, which is a key requirement for achieving Good Environmental Status in marine ecosystems. It is currently well acknowledged that the monitoring of chemical concentrations alone is not sufficient to protect populations since considering only a tiny number of substances while a myriad of others is left unnoticed. In addition, the hazards related to contaminant mixtures remain undetected. Examining biological effects provides an understanding of the impact of the multiple mixed effects of contaminants on marine biota. Recently, the Helsinki Commission (HELCOM) sub-team focusing on biological effects of hazardous substances (EG Haz BE) has been seeking to develop and support the implementation of effect-based methods (EBM) in the integrated chemical-biological monitoring and assessment frameworks for the Baltic Sea region. These efforts resulted in developing a regional platform for EBM using support for several joint activities. From the Baltic Sea viewpoint, this cluster of new activities involving the HELCOM EG Haz BE as the core group has significant potential to improve monitoring and assessment strategies and to increase understanding of the importance of EBM in current monitoring programmes. The cluster of activities establishes direct contact with different types of stakeholders to evaluate their needs and barriers in addressing mixed effects of contaminants. The scene will thus be set for recommendations on harmonised procedures to be implemented at municipal, national, or regional levels with the aim of establishing foundations for a long-term practical approach to addressing the pollution impact on the Baltic Sea ecosystem.

#### **4.08.P-Mo364 "Socioeconomic Dynamics of Sunscreen Pollution from Coastal Tourism: A Comprehensive Assessment of Beachgoer Habits and Preferences in Southern Spain"**

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To address the challenges of marine and coastal pollution, it is essential to gain an understanding of the pressures, and impacts associated with the activities we aim to manage. In the context of coastal tourism, an understanding of the effects on marine ecosystems become paramount for formulating sustainability objectives. A pertinent example is the case of sunscreens. Coastal pollution attributed to sunscreen has emerged as a significant environmental concern, prompting the need for comprehensive strategies. While most studies have followed an experimental approach in controlled environments to analyze the effects, there is still a considerable lack of works that have considered the use patterns of these products on beaches to assess sunscreen pollution. The delineation of interrelationships and socio-economic dynamics (encompass the description of user behavior and their perceptions of resultant issues) provide the necessary context for assess both the current and potential impacts of sunscreen-related pollution.

In this work, we designed and applied a survey of beachgoers to analyze their habits, sunscreen consumption, input to coastal waters, and willingness to pay for eco-labeled alternatives. The study focuses on two urban beaches in the highly popular tourist destination of southern Spain: La Caleta in Cadiz (n=205) and La Bajadilla in Malaga (n=198). Our findings revealed significant disparities between the two case studies, both in terms of the percentage of beachgoers using sunscreen (97.1% and 89.4% respectively) and the volume of sunscreen used (7.9 and 11.9 ml per application and person respectively). While these amounts were below the recommended maximums, they surpass estimates from theoretical studies. This result in a total sunscreen input into coastal waters of 76.3 and 109.3 ml per user during the summer season in La Caleta and La Bajadilla, respectively. On a per-person basis, respondents' average willingness to pay was 11.5€/250ml and 14.2€/250ml, respectively. Despite the incipient perception of the problem, the results indicated a positive inclination towards eco-labeled alternatives.

The divergent outcomes from two ostensibly similar beaches underscore the necessity for a more thorough and on-site characterization of beachgoers and their habits to advance our understanding of the potential impact of sunscreens on marine ecosystems, facilitating effective and sustainable tourism management.

#### **4.08.P-Mo365 Characterization of scrubber water discharges from ships using advanced suspect and target screening strategies**

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While shipping is acknowledged as an energy-efficient transport mode, ship engine combustion produces pollutants like particulate matter, carbon dioxide (CO<sub>2</sub>), nitrogen and sulphur oxides (NO<sub>x</sub>, SO<sub>x</sub>) that are emitted to the atmosphere. Currently, many shipping companies have installed exhaust gas cleaning systems, also known as scrubbers, on their vessels, to comply with the restrictions set by the International Maritime Organization (IMO) concerning SO<sub>x</sub> emissions. During the scrubbing process, the exhaust gas is driven through a fine spray of water which readily dissolves SO<sub>x</sub> so that levels are sufficiently reduced in air emissions. However, the waste stream generated, known as scrubber water, contains a mixture of contaminants that are directly discharged into the marine environment. Despite the widespread adoption of scrubbers, there is limited information on the chemical composition and environmental risks associated with scrubber water discharges. Previous research has identified polycyclic aromatic hydrocarbons (PAHs) and metals as prevalent pollutants in scrubber waters, with alkylated PAH derivatives anticipated to be present at higher concentrations than their parent compounds. To address this gap, in this study a suspect screening based on gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) was used to identify the most relevant PAHs and alkyl-PAHs in scrubber waters. Subsequently, a target method was developed based on headspace solid-phase microextraction (HS-SPME) coupled to GC and tandem mass spectrometry, to identify PAHs and alkyl derivatives in scrubber waters from an on-board case study in a container ship, sailing from the North Sea to the Eastern Mediterranean, with the aim to pinpoint key substances acting as markers for scrubber water contamination in marine ecosystems. The suspect screening successfully identified 7 PAHs and 12 alkyl homologues. The most prevalent were naphthalene, phenanthrene and fluorene derivatives covering from one to four (C1-C4) degrees of alkylation. Notably, methyl (C1) derivatives were particularly prominent among identified alkyl homologues. In the results from the on-board sampling campaign, sea waters showed minimal detection of target compounds while scrubber waters exhibited high concentrations, particularly for naphthalene, phenanthrene and their alkylated derivatives, emphasizing the environmental significance of these compounds in scrubber water discharges.

#### **4.08.P-Mo366 Quantitative estimation of drivers for ecotoxicological effects of scrubber water discharge to the marine environment**

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Global trade is heavily reliant on ocean shipping with around 90% of traded goods being shipped in the maritime sector. The sector is predicted to triple by 2050. While the Nordic Region are front runners in efforts to reduce the environmental impact of maritime activities in line with Sustainable Development Goals such as SDG 12 and 14, there is still grounds to be covered with regards to identifying significant contributors to ecotoxicological effects of discharges related to technologies set to reduce the maritime emission of SO<sub>x</sub> e.g. scrubber technology. Discharges from both open and closed scrubber systems emit substances of environmental concern such as polycyclic aromatic hydrocarbons (PAH), oil residues and heavy metals. With increasing number of vessels equipped with scrubber technology the concern increases. The inherently complex nature of the mixture makes it challenging to quantitatively assess the individual contribution to the overall toxicity, making targeted actions to minimize environmental impact difficult for both operators and regulators (e.g. OSPAR and IMO). For complex industrial discharges the US-EPA developed Toxicity Identification Evaluation (TIE). The approach utilizes a series of physical and chemical process operations allowing a step-wise evaluation of the residual toxicity following each step. Hereby, identifying fractions that contribute/dominate the toxicity (e.g. metals, volatile compounds, non-polar or polar organic compounds). The approach has so far not been applied to scrubber water. The poster will present results on 1) the utilization the TIE approach to identify main contributors to the whole effluent toxicity 2) evaluation of pH on the bioavailability of contributors identified 3) evaluate dilution need and the potential reductions (based on TIE) required to comply with current guidelines inside/outside of



emission control areas according to IMO's MEPC. The overall goal is to create a scientific basis for targeted approaches to minimize the environmental impact of scrubber water discharges and the feasibility of reaching current targets of MSFD and evaluating this end-of-pipe technology in a broader context of emerging concepts such as the Safe-and-sustainable-by-Design.

#### **4.08.P-Mo367 Generating inputs for the calibration of oil spill effects models: Filling data gaps for marine and freshwater species of importance and application of oil spill biomonitoring system**

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Through the program Multi-Partner Research Initiative (MPRI), Natural Resources Canada has funded a 4-year project focussed on testing, evaluating, adjusting, coupling, and validating a combination of existing models (oil solubility model, target lipid model (TLM), PETROTOX, and SIMAP) to predict potential environmental impacts of spilled oil. We will address key knowledge gaps related to complex interactions of confounding abiotic (photomodification) and biotic (species sensitivity) variables using a combination of high-resolution chemical analyses, state-of-the-art ecotoxicological applications and oil spill fate and effects models. We will utilize existing and acquire new data on detailed oil chemistry and ecotoxicology considering important abiotic and biotic variables. Comprehensive high-resolution chemistry data (GC×GC-FID) on crude oils (fresh and weathered) will be used to assign hydrocarbon blocks and predict toxicity using existing models, which will be validated with targeted experiments using whole oils. We will focus primarily on sensitive life stages of aquatic species to benchmark against existing datasets applied in oil spill models. Already existing data sets on oil high-resolution chemistry and ecotoxicity testing (*Skeletonema pseudocostatum*, and *Calanus finmarchicus*) for >20 oils and data with single PACs for several marine species will be expanded with data on additional freshwater and marine species and will include sublethal endpoints including physiological performance (e.g., metabolic rate and cardiac output) and biomarkers of exposure in lumpfish (*Cyclopterus lumpus*). The lumpfish biomarkers will be validated in a mesocosm and field exposure, using a newly SINTEF-developed technology for housing lumpfish embryos for in-situ exposure and effects assessment. Through this project, this technology will be intercalibrated and further developed for the use in the event of future accidental or intentional research-related oil spills in the marine environment. In total, this project will provide the basis for improved reliability of risk, impact, and damage assessment models, as well as NEBA-based decision making, in the event of oil spills in sensitive marine and freshwater environments.

#### **4.08.P-Mo368 Risks of Environmental Impacts of Accidental Spills of Ammonia to the Marine Environment**

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Regulations for fuel usage in maritime traffic by MARPOL are setting global standards that aims at reducing air pollution, reducing carbon emissions, and increasing energy efficiency. However, in the event of an accidental acute spill, how does conventional fuel oils compare to green ammonia in terms of potential environmental impact? We explored this challenge by a combination of an experimental ecotoxicology and a modelling approach. First, we assessed the acute and sub-lethal toxicity of fuel oils and ammonia to Atlantic cod (*Gadus morhua*) embryos. Atlantic cod embryos were exposed for 4 days starting 3 days after hatch, and after exposure, the embryos were transferred to clean sea water until 3 days post hatch. Survival and hatching were monitored daily, and at the end of the experiment, larvae were imaged for assessing larvae morphometry and potential deformations. Second, we simulated acute spills of the fuels using the Dose-related Risk and Effects Assessment Model (DREAM) and the Oil Spill Contingency and Response model (OSCAR) to predict spreading and estimate the risk of toxicity to pelagic marine organisms. Ammonia caused acute toxicity to cod embryos; however, no indications of delayed toxicity were observed. No additional mortality was observed during the recovery period, and the surviving larvae displayed no deformations. Fish exposed to petrogenic fuel oils, however, displayed both acute toxicity as well as clear signs of deformations. DREAM and OSCAR simulations suggests that most of the ammonia reached the surface and evaporated, but some spreading of ammonia as ammonium generated a plume which displayed concentrations exceeding predicted no-effect concentrations (PNEC). Spills of marine gas oil, very low sulphur fuel oil and heavy fuel oil behaved differently. MGO, like ammonia, spread in the water column, however, substantial fractions also evaporated, biodegraded, surfaced, and reached shorelines. Most of the heavy fuel oil and very low sulphur fuel oil, however, ended up on the surface and were carried by waves and to shorelines, and very little of the mass ended up in the water column. The MGO had a lower PNEC than ammonia, so the total volumes of water containing concentrations exceeding the PNEC was higher for MGO than ammonia. For the VLSFO and HFO, insignificant volumes of water exceeded PNEC, due to the fate outcomes discussed above.

#### **4.08.P-Mo369 Risk-based Approach for Regulation of Marine Pollution**

**Kirit Wadhia**, *Environmental, NOV Inc, United Kingdom*

It is apparent that regulations worldwide exist for management of marine pollution dealing with different industry sectors. There is disparity between the different frameworks employed and even within. Criteria for different sectors require particular and specific considerations. Concern of marine pollution impinges on the change pertaining to physical, chemical, and biological state of the water bodies and coastal areas. The 1982 UN Convention on the Law of the Sea defined marine pollution as: "the introduction by man, directly or indirectly, of substances or energy into the marine environment ... which results or is likely to result in such deleterious effects as harm to living resources and marine life." (UNCLOS, 1982). The most significant threat is the potential impact on marine wildlife and ecosystems. Chemical pollution in general can emanate

from a range of sources including crude oil and other petroleum products, antifoulants, pesticides, pharmaceuticals and personal care products. In context of the petroleum sector industrial discharge (i.e. produced water) containing chemicals from anthropogenic and natural sources is of primary concern. The offshore oil and gas industry thematic strategy (Offshore Strategy) sets the objective of preventing and eliminating pollution and taking the necessary measures to protect the OSPAR maritime area against the adverse effects of offshore activities so as to safeguard human health, conserve marine ecosystems and, when practicable, restore marine areas which have been adversely affected. Risk assessment involves evaluation taking into consideration the potential direct and indirect consequences. It involves comparison of the exposure of the ecosystem to chemicals of pertinence & concern with the sensitivity of the ecosystem for these chemical through the specific exposure-route. Perspective pertaining to the regulatory framework with insight of developments, considerations and progress concerning risk-based approach for the management of produced water discharges is the focus of this conveyance.

Reference: UNCLOS. (1982). United Nations Convention on the Law of the Sea. Available at: [https://www.un.org/Depts/los/convention\\_agreements/texts/unclos/UNCLOS-TOC.htm](https://www.un.org/Depts/los/convention_agreements/texts/unclos/UNCLOS-TOC.htm)

#### **4.08.P-Mo370 A New IOGP Report on Fate and Effects of Naturally Occurring Substances in Produced Water**

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Produced water, generated during the production of oil and gas from offshore wells, is the largest continuous operational waste stream discharged to sea by the offshore oil and gas industry. Produced water contains a complex mixture of dissolved and particulate organic and inorganic substances including naturally occurring substances (NOS) from geological formations such as hydrocarbons, metals, and chemicals intentionally added during production processes. Some components of produced water have hazardous properties including environmental persistence, toxicity, potential for endocrine activity, and potential to bioaccumulate in marine organisms. Consequently, produced water discharges are heavily regulated.

The International Association of Oil and Gas Producers (IOGP) has previously published technical reports describing the fate and effects of NOS in produced water. However, in the two decades since publication, the scientific understanding of the fate and effects of NOS in produced water has expanded considerably. In order to produce an up-to-date Report, a structured review of evidence in the scientific literature was performed, and an overview of the composition, fate, and effects of NOS in produced water has been produced. The poster presents the main conclusions of the Report, describing the constituents present as NOS in produced water, their fate and effects considering both substance-based and whole effluent testing, and field-based monitoring and surveillance activities. It is envisaged the work will inform relevant practitioners of the environmental risks from produced water discharges to the marine environment.

#### **4.08.P-Mo371 Analysis of emerging contaminants in ship greywater through liquid chromatography and a wide scope target high resolution mass spectrometry screening method**

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Shipping has long been recognized as an energy-efficient transport medium for moving freight. However, the increase of maritime traffic has led to the continuous discharge of substantial volumes of wastewater into the marine environment, posing potential threats to vulnerable ecosystems. Greywaters, among the various ship-released wastewaters, constitutes a significant portion of the total volume of water produced. Currently, the release of greywater from ships lacks comprehensive regulation in numerous sea regions. Moreover, these discharges may contain a diverse array of chemical contaminants, including pharmaceuticals and personal care products, and their composition may be influenced by on-board activities. Since ship greywaters are more concentrated than land-based wastewaters, it is of outmost importance to perform a comprehensive chemical characterization of greywaters emitted from ships to fully assess the potential environmental and human health risks associated with their discharge to the marine environment. This study focused on using the benefits of wide scope target screening by high-resolution mass spectrometry (HRMS), using an Orbitrap Exploris<sup>TM</sup> 120 instrument, for the holistic characterization of ship greywaters. Samples were provided by the BSAG, who collected greywaters from cargo ships docked at Helsinki port in Finland, as part of the Horizon 2020 EMERGE project (Grant agreement ID: 874990). The methodology employed encompassed information for more than 700 compounds, including the exact mass of precursor and fragment ions and their retention time, allowing for the identification of contaminants in the samples. With this holistic approach, 88 compounds were tentatively identified, with 44 fully validated and quantified using analytical standards. A semi-quantification tool was used to estimate the approximate concentration of the remaining 44 substances. The compounds identified spanned various chemical classes, including pharmaceuticals (e.g. antihypertensives, analgesics and antidepressants), stimulants (e.g. caffeine and metabolites), tobacco and food related products, personal care products, UV filters, surfactants, perfluoroalkyl substances, plasticizers and flame retardants, among others. This comprehensive analysis provides valuable insights into the composition of ship greywaters, facilitating a better understating of potential environmental implications and human health risks associated with their discharge

#### **4.08.P-Mo372 Persistent Organic Pollutant Accumulation in Pacific Abyssal Plain Sediments and Biota: Implications on Sources, Transport, and Deep-Sea Mining**

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Despite its vast size, ecological, and economic importance, the deep sea is one of the least understood ecosystems on Earth. While much remains to be discovered, researchers have established that the deep sea is being exposed to numerous anthropogenic stressors, one of which is chemical pollution. Persistent organic pollutants (POPs), defined by their persistence in the environment, bioaccumulation, and high toxicity, are continually discharged and transported into the deep sea despite protections under global and regional conventions. Few studies have provided insight into POP transport processes and accumulation in the deep sea and none to our knowledge have examined POPs at the seafloor of the Pacific abyssal plain; an area currently being targeted for deep-sea mining and previously assumed to be too far removed to be affected by POPs. Here, we provide baseline data on POPs in deep-sea fish from a region of the deep abyssal plain targeted for deep-sea mining. All congeners of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and multiple per- and polyfluoroalkyl substances (PFAS) were measured in sediment, fish muscle, and fish liver of a deep-ocean predator and scavenger, *Coryphaenoides sp.*, to provide information on sources, transport, accumulation, and contributions of legacy versus emerging (e.g., unintentional PCBs) POPs. Here, we provide the first evidence of PFAS, PCBs, and PBDEs in sediment and biota in this region of the Pacific abyssal plain. Preliminary results show that PCBs were more evenly distributed over the sampling region, while PBDEs and PFAS had patchy distributions and concentrations. Unintentional PCBs (e.g., PCB 11, a component of pigments) significantly contributed to sediment and muscle tissue concentrations, suggesting unregulated PCB releases are accumulating in the deep Pacific Ocean. PFAS was detected in one sediment sample and all biota at levels as high as those found in freshwater ecosystems. Results suggest that the flux of carrion, organic matter particles and vertically migrating species may present an important transport process for POPs that result in patchy contaminant distributions and differences seen in scavenging biota versus sediment. However, for PCBs, with a longer production duration, sinking particle transport may be a more important driver, with raining particles (e.g., POM and plastics) laden with adsorbed PCBs, resulting in a more uniform distribution across this region.

#### **4.08.P-Mo373 What is in the water? Evaluating patterns of organic chemicals at different depths on a transect across the North Pacific Ocean**

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We aimed to unravel patterns of organic pollutants in water samples from different depths on a transect across the North Pacific Ocean, from Vancouver to Singapore, in 2019 in the frame of the project Micro-Fate. During leg 268/3 of the German Research Vessel SONNE, 45 large-volume seawater samples (60 L) were collected using two methods. On the one hand, deep waters were grab sampled using the Niskin bottles attached to a CTD-Rosette, with which water from a) the bottom (close to the sea floor, at around 5000 m depth), b) 2500 m depth, c) 300 m depth and d) at the depth of the Chlorophyll maximum, were sampled at 8 stations. On the other hand, surface water was sampled using the ship's rotatory pumps, at the same stations where the CTD-Rosette was deployed and while steaming (7 samples). Each sample was extracted on board using a large-volume SPE (LV-SPE) device, equipped with cartridges filled with Chromabond® HR-X sorbent. To our knowledge, this is the first time that this LV-SPE approach has successfully been applied in an oceanic sampling campaign, and some adjustments and challenges due to the salinity and the movement of the ship needed to be solved *in situ*. After each extraction, 1 L of LC grade water was circulated through the cartridges to remove the salts, and they were kept cold at 4°C on board. After finishing the sampling campaign, the cartridges were shipped cooled to our laboratories, dried using nitrogen, freeze-dried and stored at -20°C until elution. They were eluted with a sequence of solvents, following Schulze et al.

[doi.org/10.1016/j.scitotenv.2016.12.140], and the volume was reduced, adjusted to 5000-fold concentrated extracts relative to the initial volume of water. The analysis was carried out using LC-HRMS Orbitrap Q-Exactive, Thermo Fisher, and MzMine and MzQuant were used to evaluate 600 compounds. The preliminary results showed 140-150 potentially quantifiable compounds present in the samples, currently under evaluation according to our established quality assurance/quality control (QA/QC) procedures. Final results will be disclosed at the SETAC Europe 34th Annual Meeting.

#### **4.08.P-Mo374 Effects of Particles from Road Tunnels Construction on Post-Smolts Atlantic Salmon**

**Samantha Eslava Martins, PhD<sup>1</sup>, Maria Christou<sup>2</sup>, Tania Gomes<sup>1</sup>, Ana Catarina Almeida<sup>2</sup>, Steven Brooks<sup>2</sup>, Maria T. Hultman<sup>2</sup>, Simon Rodriguez<sup>2</sup>, Joachim Johansen<sup>2</sup>, Adam Lillcrap<sup>1</sup>, Emelie Forsman<sup>2</sup>, Lene Heier<sup>3</sup> and Sondre Meland<sup>2</sup>,** (1)Norwegian Institute for Water Research (NIVA), Oslo, Norway, (2)Norwegian Institute for Water Research (NIVA), Norway, (3)Norwegian Public Roads Administration, Norway

Suspended solids (SS) in coastal ecosystems may arise from natural or anthropogenic sources, the latter including emission of particles from road construction. When tunnels are constructed in Norway, drilled and blasted bedrocks disposed in nearby

fjords may increase the concentration of particles in suspension as well as associated chemicals. Some fjords are used by salmon as a migration corridor during their life cycle. In this study, the impact of particulates on post-smolts Atlantic salmon (*Salmo salar*) was investigated. Fish (mean weight at test start: 130 ± 14 g) were exposed to fine particles (< 100 µm) from the construction of a tunnel, at nominal concentrations of 4, 40 and 400 mg SS/L for 21 days, followed by 7 days of depuration. A control- and a naturally eroded particles (NEP, 400 mg/L) group, collected at a reference river, were run in parallel. A Recirculating Aquaculture System (RAS) with a dosing system was installed to reduce particle loss due to sedimentation. Analysis to elucidate the effects of these particles at histological, osmoregulatory, immunological and genetic levels are underway, but the preliminary results show that salmon exposed to the highest concentration have reduced growth compared to fish exposed to NEP. In addition, surface and slow swimming behavior, caudal fin deformities, empty intestinal content and inflamed gallbladder were observed in some individuals exposed to the highest concentration. This study will increase knowledge on the sublethal effects resulting from the exposure to particles from tunneling activities on Atlantic salmon and their ability to recover after dumping is ceased, providing results that can support the strategy for tunneling construction particles disposal in water systems. The joint road and rail project Arna-Stanghelle is a collaborative research between the Norwegian Public Roads Administration (main funding), Bane NOR, and NIVA.

#### **4.08.P-Mo375 Metal bioaccumulation, trophic dynamics, and risk of consumption of various marine fish species from the Indian River Lagoon (Florida, USA)**

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Trace metals bioaccumulation in the marine biota can pose great environmental risk to the aquatic ecosystem once reaching toxic levels. Metal measurements among a variety of marine fish species considering a range of trace elements is a research subject still expanding; namely those focusing on species with dissimilar trophic behaviour. Therefore, this study aims to generate in-depth information on bioaccumulation, trophic dynamics, and risk of consumption by humans using a suite of trace metals considering recreational and economic important fish species (*Sciaenops ocellatus*, *Lutjanus griseus*, *Ariopsis felis*, *Centropomus undecimalis*, *Bairdiella chrysoura*, *Cynoscion nebulosus*, *Archosargus probatocephalus*, and the shark *Sphyrna tiburo*) collected from impacted sites in the estuary Indian River Lagoon (Florida - USA). Notably, trace elements (THg, MeHg, As, Se, Pb, Cd, V, Nd, La, and Ce) known by their significant toxicity are being measured in fish's liver and muscle using inductively coupled plasma mass spectrometry (ICP-MS); and metal concentration in each species will be linked to their trophic dynamics and feeding behavior. Additionally, the processes of biodilution and biomagnification will be evaluated. Differences in total metal concentrations among the fish species selected are observed where biological features (size, age) and ecological behaviour (trophic position and diet sources) may be explanatory factors of these results. Biodilution is observed for most trace metals. Correlating the contamination level of each fish species with their nutritional ecological habits might help to identify which trophic level is most likely to be impacted. By revealing this novel information, we hope to provide original data and tools to strengthen our environmental protection awareness and capabilities, ultimately guiding us toward more informed and effective management practices.

#### **4.08.P-Mo376 Risk assessment of Potential toxic Elements in Marine coastal areas of Campania Region (Southern Italy)**

*Luisa Parrella*<sup>1</sup>, *Simona Schiavo*<sup>2</sup>, *Sara Accardo*<sup>3</sup>, *Maria Rita Montereali*<sup>4</sup>, *Giovanna Armiento*<sup>5</sup> and *Sonia Manzo*<sup>6</sup>, (1)ENEA PORTICI Research Center, Italy, (2)National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Italy, (3)University Parthenope, (4)ENEA Casaccia Research Centre, Italy, (5)ENEA, (6)ENEA CR Portici, Italy Addressing cumulative multi-hazards related to pollution of marine coastal areas (MCA) in a climate change perspective is today a real challenge that needs interdisciplinary approach to enhance our ability to protect and preserve ecosystems in the face of complex and interrelated threats.

MCAs are exposed to different anthropogenic impacts (e.g., coastal land use, maritime activities, river inputs) and are sensitive to climate change consequences (e.g., acidification, loss biodiversity, costal erosion, ecc) that can contribute to a deterioration of coastal environmental quality.

An approach of multi-risk assessment was applied to MCAs of Campania Region (Southern Italy), in order to evaluate the risk of diffuse Potential Toxic Elements (PTEs) pollution and their ecotoxicological effects on one or more component ecosystems in a contest of climate change. This study is part of "ISSPA" Project supported by PO FEAMP (Fondo Europeo per gli Affari Marittimi e la Pesca) Campania 2014-2020.

Chemical monitoring data was collected between September - October 2020 and June - July 2021 in 27 stations of Campania coastline. The occurrence of PTEs in water (As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, U, V e Zn) and sediment (As, Be, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, U, V, Zn, Al e Fe) was investigated and a comparison of potential Ecological Risk Assesment (ERA) of mixture of chemicals (As, Cd, Cu, Ni, Zn) and of observed Ecotoxicological Risk for water and sediement was conducted.

Results obtained by overlaying Ecological Risk Assessment with Ecotoxicological Risk Evaluation showed that risk was not attributable only to PTE mixture and that different pollutants could be a role in the observed toxicity. This evaluation will be useful to provide an overview of ecological and ecotoxicological risk for this particular coast and a prioritization of coastal stretches for managing diffuse metal pollution.

Also the actual environmental changes need to be considered in the ERA ecotoxicological data selection.

#### **4.08.P-Mo377 Integrated Chemical-Biological Monitoring of Pollution off Main Coastal Cities of Finland Using the Perch (*Perca fluviatilis*) as the Indicator Species**

*Ville Aimo Eemeli Junttila, Anu Lastumäki, Rami El Dairi, Katri Siimes, Tanja Kinnunen and Kari K. Lehtonen, Finnish Environment Institute (SYKE), Finland*

In Finland, the regular national monitoring of environmental chemicals in marine coastal waters has been running for a few decades based on tissue concentrations of selected organic compounds measured in the muscle tissue of perch (*Perca fluviatilis*). In 2015, the monitoring programme was supplemented by the inclusion of the biological effect parameter lysosomal membrane stability (LMS), measured from frozen liver tissue sections using the histochemical method. LMS is a widely recommended biomarker for the assessment of cytotoxic effects and indicates non-specific stress, which may be caused by a large variety of chemical contaminants. In this presentation we report the results of monitoring of tissue concentrations of selected organic chemicals such as planar and dioxin-like PCBs, DDTs, dioxins, brominated flame retardants, and mercury, in conjunction with LMS and morphometric indices measured in perch collected yearly during 2015-2022 from five monitoring stations located off the cities of Helsinki, Kotka, Pori and Vaasa along the Baltic Sea coast of Finland, known to be under marked anthropogenic influence. Statistical analyses were carried out on the data to detect changes over time at each of the study sites as well as differences between them. Multivariate analyses were performed on the chemical and biological parameters to examine relationships between them. This is the first report of a continuous integrated chemical-biological monitoring of contaminants performed in coastal marine areas of Finland and shows the usefulness of the approach in producing more reliable assessment of the contamination status of the region.

#### **4.08.P-Mo378 Assessment of 6PPD-Quinone Acute Toxicity to San Francisco Bay Delta Fish Species and Sublethal Effects to Salmonids**

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Tire and road wear particles are an inevitable consequence of tire use in automotive traffic and are of particular concern in near-urban ecosystems. A toxic transformation product of a common tire additive, N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), has been detected in water samples from San Francisco region creeks at concentrations near and above the LC50 for Coho Salmon. The presence of 6PPD-quinone in the San Francisco Bay-Delta region thus presents a potential risk to local aquatic species. This project aims to assess the toxicity of 6PPD-quinone to five species of conservation concern: Coho Salmon, Chinook Salmon, Steelhead, Longfin Smelt, and Delta Smelt. Larvae and juveniles of each species were tested for acute toxicity with ongoing investigations of sublethal toxicity. Sublethal endpoints include behavior, thermal tolerance, and swimming performance to assess potential neurotoxicological and cardio-respiratory effects of 6PPD-quinone exposure. Results suggest interspecies differences in susceptibility, with Coho Salmon and Steelhead showing acute sensitivity at 0.07 and 5.00 µg/L, respectively, while Chinook Salmon, Longfin Smelt, and Delta Smelt are acutely insensitive. Together with acute assay details, we will also present sublethal effects determined through behavioral assessments. Observed erratic behavior in exposed Chinook Salmon and Coho Salmon support the growing weight of evidence that 6PPD-quinone acts as a neurotoxicant. The results from this study will inform ongoing conservation efforts for these critically threatened species.

#### **4.08.P-Mo379 Detection of Tire Rubber Particles from a Football Field in an Urban Estuary**

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Tire particles (TPs) are one of the largest sources of microplastics to coastal systems in Norway, and understanding their fate and impact in the environment is an important prerequisite to implement measures and solutions for environmental challenges from human activities, such as developing more sustainable transport systems in the future as part of the envisioned 'green shift'. Based on recent method development to detect TPs and studies on the uptake and toxicity of TP additives, we here aim at quantifying tire particle pollution pathways in a Norwegian coastal system to evaluate different TP markers in riverine and estuarine sediment samples to elucidate the transport pathways from land to ocean in a gradient from a football field with TP granulate infill via a local creek to an urban estuary in the town of Tromsø, Norway. Samples from the football field and from the estuary were analyzed for TP using pyrolysis gas chromatography mass spectrometry (Pyr-GC/MS) using four different markers and marker combinations for styrene butadiene rubber and butadiene rubber (SBR/BR): M4 (*benzene, α-methylstyrene, ethylstyrene and butadiene dimer*) M3 (*α-methylstyrene, ethylstyrene and butadiene dimer*), VCH (*vinylcyclohexene*), and Butadienes (*butadiene dimer, SB dimer and SBB trimer*). The results show varying marker concentrations in selected tire granulate samples from the football field, where M3 and Butadienes were the most promising candidates for the quantification of rubber in the field samples. The sediment samples showed similar trends for both M3 and

Butadienes, where the mid-river station showed the highest rubber concentrations at roughly an order of magnitude lower concentrations than the rubber granulate on the football field. These preliminary data will serve further refinement of rubber detection methods and guidance for more comprehensive field sampling to distinguish granulate runoff from potential background contamination from local traffic.

#### **4.08.P-Mo380 Assessment of Microplastics in the Sediments around Hywind Scotland Offshore Wind Farm**

**Andy Booth, PhD<sup>1</sup>, Stefania Piarulli<sup>1</sup>, Lisbet Sorensen<sup>1</sup>, Stephan Kubowicz<sup>2</sup>, Helene K Vrålstad<sup>3</sup> and Kari Mette Murvoll<sup>3</sup>,**  
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Leading edge erosion of turbine blades is thought to be a primary source of MP emissions to the marine environment. Leading edge protection (LEP) materials are increasing being used to protect the coatings and reduce damage to the main rotor blade, but might also represent a source of MP emissions. Estimates indicate that up to 200 g of coating material can be lost in this way per turbine per year. Given the higher density of rotor blade coating materials than seawater, released MP are expected to sink to the sediments and accumulate around the OWF infrastructure. Methods for the general extraction, clean up and analysis of thermoplastic MPs from sediments are well established, this has not been completed for OWF coatings. To ensure OWF particles can be reliably identified and quantified, there is a need for chemical fingerprinting and verification of extraction and sample clean up techniques.

Here, we present an approach for the quantification and characterisation of microplastics (>300 µm) in sediments collected from the Hywind Scotland floating OWF in Scotland, with a focus on distinguishing MP derived directly from OWF infrastructure (i.e., rotor blades coatings and LEP) from background levels of conventional thermoplastic MPs (e.g. PE, PP, PS, PET, PVC). Reference materials produced from the different coating layers and LEP material were produced and used to generate library infrared spectra and mass spectra. The reference materials were also used to assess if the density separation (ZnCl<sub>2</sub>) and filtration approaches used in the pre-processing and purification steps caused damage to the particles. Results suggested the LEP material was unaffected by the sample processing, but fragmentation was observed for the two coating layers studied, leading to an increase in the number of particles and a decrease in weight due to loss of particles <300 µm. Finally, the methods were applied to remove biogenic and mineral particles, isolating MP from the real sediment samples (3 x 100 g of sediment from each of the 15 sampling locations). After isolation, the particles were evaluated using microscopy and a total of 30 candidate MP particles were collected and sent for detailed chemical characterisation by Fourier transform infrared spectroscopy. Twenty of the particles were confirmed to be MP, but none had polymer compositions matching the coating and LEP materials, instead representing common thermoplastics in the form of flakes, films, fragments and filaments.

#### **4.08.P-Mo381 A History of Microplastic Pollution in UK Salt Marshes**

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The amount of plastic pollution in the environment has increased exponentially since its mass production began in the 1950s. As the vast majority of plastic cannot biodegrade, it instead tends to degrade slowly by mechanical and chemical processes, producing microplastics (MPs, < 5 mm) that are found in all areas of the globe. There is increasing concern about the potential negative impacts of MPs on the environment. Considerable research has been performed concerning MP pollution in aquatic and coastal environments but the amount of plastic in UK salt marshes is yet to be quantified.

Salt marshes are types of coastal wetland with a unique range of flora and fauna; they provide coastal protection and many other ecosystem services, including sequestering 'blue' carbon. Sediment deposited by high tides, as well as growth and decay of plants, allows vertical growth of salt marshes. The sediment deposited may contain pollution such as plastic, so it is possible that these coastal wetlands act as a plastic sink, accumulating MPs over time. However, there are very few studies of MPs in salt marshes, and even fewer that incorporate sediment dating using short-lived radio-isotopes (<sup>210</sup>Pb, <sup>137</sup>Cs, <sup>241</sup>Am) to estimate when the plastic pollution entered the environment.

In this study, 29 salt-marsh cores from around the UK coastline were dated using <sup>210</sup>Pb, <sup>137</sup>Cs and <sup>241</sup>Am. Chronologies were constructed using the Bayesian Plum model. A method for the extraction of MPs from these cores and their identification was developed in order to create a history of MP pollution in UK salt marshes, giving insight into the types and quantities of plastics present.

To extract MPs, organic matter in salt marsh sediment was first digested using 30% H<sub>2</sub>O<sub>2</sub> in an ice bath, rather than the commonly used method of digesting with 30% H<sub>2</sub>O<sub>2</sub> and heating to 70°C. MPs were then extracted from the sediment using density separation with LST FastFloat. This was followed by a combination of Nile Red staining, fluorescence microscopy and Raman spectroscopy to identify the types of plastic present.

This study is the first of its kind conducted on UK salt marshes. The novel extraction methodology will allow a more accurate count of the number of MPs present in sediment. The accurately dated cores will allow a detailed history of MP pollution in salt marshes to be constructed.

#### **4.08.P-Mo382 Microplastic Pollution Along the Mediterranean Coast of Turkey: Impacts on Loggerhead Turtles (*Caretta caretta*) Nesting Environments and Coastal Ecosystems**

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Plastic pollution, a global environmental concern, leads to significant amounts of microplastics (MPs) in oceans due to the breakdown of larger plastics worsened by improper disposal practices. The presence of microplastic pollution on beaches, particularly in areas close to the ocean, clearly indicates more significant plastic pollution issues. Due to their high exposure, loggerhead turtles are crucial biomarkers for monitoring plastic pollution in the Mediterranean coastal area. This study aimed to determine the level of microplastic exposure faced by *Caretta caretta* turtles along their nesting route and hatching. Samples were collected during the morning and night at the same points to observe the effect of wind direction on MP distribution. We characterised microplastics collected from a protected nesting area based on their colour, shape, size, and polymer type. The sand samples were isolated using density separation and oxidation procedures and analysed visually with stereomicroscopy and chemically with Fourier Transform Infrared Spectroscopy. The preliminary results identified 16 types of microplastics, with common types across all samples being Polyethylene (PE), Polypropylene (PP), Cellulose, High-density Polyethylene (HDPE), and Tencel. The region's high urbanisation and increased tourism contribute to the diversity and abundance of MPs. Various plastic types, including polyethylene-vinyl acetate (PEVA), low-density polyethylene (LDPE), and polystyrene (PS), were found in both day and night samples, reflecting their widespread use. The research highlights the concern of MP accumulation on nesting sites in the Mediterranean Sea of Turkey, particularly impacting marine turtles' incubating environment. MPs affect species indirectly in coastal environments and potentially affect marine turtles' nesting success. During subsequent phases of the study, the trajectory of microplastic pollution along the coastline will be determined, and the factors influencing this pattern will be investigated. Further research endeavours may explore the health risk assessment by microplastics on loggerhead turtles by conducting a thorough investigation into the various adsorbed chemicals, additives, and other parameters that may affect the risk assessment to examine how these factors contribute to the endangerment of this species.

Keywords: microplastic, Mediterranean, loggerhead turtles, plastic, pollution.

#### **4.08.P-Mo383 Developing a rapid assessment methodology for mangrove plastic contamination by coupling image analysis and machine learning**

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With the global environmental plastic waste burden increasing exponentially, it is urgent to develop rapid methodologies for assessing the plastic contamination of critical ecosystems. This work develops and validates an innovative methodology for identifying macroplastic pollution in mangrove forests, using a machine learning approach. Plastic monitoring is challenging in large, complex areas such as mangroves which are known to be areas of high plastic waste accumulation. Here, we used the You Only Look Once (YOLOv5) object-detection algorithm, to test whether it can detect and categorise macroplastics from video footage collected in more complex mangrove ecosystems. Video transects were collected from 3 different mangrove areas in the Galapagos Islands and 6 in Guayaquil, Ecuador, using low-cost cameras such as GoPros and smartphones. Footage collected over measured transects (ranging from 10 – 570.8 m) labelled using VIAME free software (Video and Image Analytics for Multiple Environments), by annotating the location and category of plastics (*bag, bottle, rope, packaging* and *ni* - non-identifiable) in 18 videos, totalling 21,881 frames. Labelled data was exported and uploaded to the NERC Earth Observation Data Acquisition and Analysis Service (NEODAAS) MAssive GPU for Earth Observation (MAGEO) system where it was extracted, separated into individual frames, augmented, and formatted for use with YOLOv5. For model training, the frames were split into training, validation, and test sets with different mangroves and filming conditions represented across each. The preliminary test data was 1,825 frames, and the remaining images (20,056) were split into training (80%) and validation (20%) datasets. Initial results using validation data demonstrated YOLOv5 was successful at the detection of *bag* (83%), *packaging* (88%), *rope* (72%), *bottle* (77%) and *ni* (76%). Further analysis will include trialling alternative model and image modifications to increase accuracy. Next steps are to trial modifications in the model and on images to booster accuracy. It is expected that this innovative and collaborative rapid assessment tool provides timely data on plastic contamination in large, complex, and difficult to access ecosystems such as mangroves. This will enable future identification of contamination hotspots and inform monitoring and mitigation strategies.

#### **4.08.P-Mo384 Investigations of Microplastics in Surface Water of Tokyo Bay using a Novel Automated Microplastic Sample Preparation System**

*Yutaka Kameda and Emiko Fujita, Chiba Institute of Technology, Japan*

Microplastics (MPs) in surface water, including rivers, lakes, and oceans, are increasingly recognized as a parameter for general water quality monitoring in various countries. Currently, numerous research efforts employ pretreatment methods recommended by the National Oceanic and Atmospheric Administration (NOAA). However, these methods are known to be comprehensive, labor-intensive, and time-consuming. As a result, analytical operators require techniques to minimize contamination during pretreatment, maintain the properties of MPs, and ensure the extraction of all MPs from water samples. Additionally, they may need to modify their procedures in specific cases, such as incorporating optional treatments like the Fenton reaction or digestion by sodium hydroxide to analyze MPs in organic-rich water samples. To enable precise analysis of

MPs with high efficiency, particularly for operators with limited experience, a novel automated microplastic sample preparation system (MAP-100, Shimadzu corporation) has been developed in Japan. This system can extract fragment-type MPs larger than 300 $\mu\text{m}$  from suspended matter in water samples. The process involves collecting MPs in surface water using nets such as neuston nets. Operators then gather suspended matter, removing matrices larger than 5 mm through sieves. The suspended matter smaller than 5 mm is placed in a small strainer of the system, which can then automatically perform digestion by hydrogen peroxide and density separation by sodium iodide in one continuous tube. In recovery tests, where commercial polyethylene microplastics larger than 500  $\mu\text{m}$  were added to ultrapure water and actual suspended matter from a river, the system demonstrated recovery rates of 99.0% and 93.3%, respectively. We also compared microplastic concentrations, polymer types and their size distributions in Tokyo bay between by using ordinary manual pretreatment and by using MAP-100. The fragment-shaped microplastic concentrations, polymer compositions and size distributions by MAP-100 were comparable to those by manual pretreatment. On the other hand, fabric microplastics by MAP were underestimated because of loss during the automated process. However, Poster will show a revised model of MAP-100 which can measure even fabric microplastics more accurately. Therefore, this device will be a useful automatic tool for the pretreatment process of microplastic analysis for any scientists in the world.

#### **4.08.P-Mo385 Development of Neural Network Models for Distributions of Plastics Patches in the Ocean by Sentinel-2 High-resolution Data**

*Yutaka Kameda and Emiko Fujita, Chiba Institute of Technology, Japan*

Microplastics (MPs) are widespread in the surface water of boundless expanses of the ocean. However, MPs range from less than 1 $\mu\text{m}$  to 5 mm, and their analytical and sampling methods have not been developed nor integrated globally. Moreover, recent reports suggest that large MPs, such as macro MPs or meso MPs, aggregate in surface layers of the ocean, floating with various debris as big islands or long lines accumulated by tides. It is also known that their distributions in the ocean change dramatically. Though these results indicate that the size and distributions of MPs in the ocean are very complex, it is important to reveal time trends in the geographical distributions of these plastic patches to estimate their ecological risk and control their concentrations. However, it is challenging to monitor their distributions by general grab sampling of MPs samples from the ocean.

In this study, methods for estimating seasonal geographical distributions of plastic patches in the ocean are developed using satellite data and generic models. We used four bands of spectral data from Sentinel-2 satellites, following a previous report by Biermann et al. We also used two indicators, the Floating Debris Index (FDI) and Normalized Difference Vegetation Index (NDVI), to distinguish plastic pixels from others. One strong point in our study is the establishment of a generic model capable of downloading satellite data from websites, easily correcting the data with Atmospheric Correction for OLI lite version 20181210.0 (ACOLITE), and calculating FDI values and NDVI values for each pixel. The model can also classify each pixel into nine materials such as plastic, ships, water, pumice released from volcanoes, sand, clouds, rocks, plants, and woods using the K-nearest neighbor algorithm in Python. However, the accuracy of the K-nearest neighbor algorithm is not high enough, approximately 60%. Further revisions, such as replacing the algorithm with neural network models, are needed. Nevertheless, this novel model will enable all users, including non-experts, to generate geographical distributions of plastic patches in widespread areas such as the Japan Sea, the Mediterranean Sea, the North Atlantic Sea, and other bays and lakes all over the world.

#### **4.08.P-Mo386 The First Volunteer Monitoring Project to Reveal Distributions of Microplastics larger than 1 $\mu\text{m}$ in Global Oceans by a Japanese Giant Ship Company**

*Yutaka Kameda, Chiba Institute of Technology, Japan*

Microplastics (MPs) are recognized as one of the pollutants with numerous environmental impacts. There are lots of studies of MPs in specific areas of the ocean with different methods and different polymers and sizes. This is the first study of distribution of MPs larger than 1 $\mu\text{m}$  in global oceans with a harmonized method. We are investigating the numbers, sizes and polymer types of MPs greater than 20  $\mu\text{m}$  in the global oceans starting from March 2020 and now started monitoring of MPs larger than 1  $\mu\text{m}$  from 2023. Sampling of MPs in the ocean was carried out by 14 volunteer ships with the cooperation of a Japanese shipping company. Two hundred samples had been collected by November 2022. Approximately 1  $\text{m}^3$  of sea water samples at depths of 3 to 14 meters were obtained from the hydrant of the ships. Particulate matters were collected by plankton nets (10  $\mu\text{m}$  mesh) on site. The filtered samples were pretreated by  $\text{H}_2\text{O}_2$  oxidative digestion and NaI density separation. The MPs polymers were characterized by a spectrum imaging method using micro-Fourier transform infrared spectroscopy. The MPs smaller than 20  $\mu\text{m}$  were measured by a spectrum imaging method using Raman microscope. The observed concentrations of MPs larger than 20  $\mu\text{m}$  ranged from 22 to 4,660 pieces  $\text{m}^{-3}$  in the ocean. Their geometric mean value was 260 pieces  $\text{m}^{-3}$ . Remarkably high concentrations exceeding 1,000 pieces  $\text{m}^{-3}$  were found at Kuroshio Current, California Current, Kamchatka current and North Atlantic Gyre. These results indicated that distribution of MPs was heterogeneous in the oceans. The median diameter of the MPs size distribution was approximately 60  $\mu\text{m}$ . The dominant polymer is polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET) and polymethyl methacrylate (PMMA). PET was the most dominant fibrous polymer. Interestingly, PMMA was detected predominantly in the Pacific Ocean. There were some PMMA hotspots in Kuroshio Current. Polymer types and shapes may be influenced by land-based input, ocean currents and wave-driven. The PMMA contamination might be unique to Asia. This project will continue several years later and can provide data on MPs abundance to further the understanding of MPs pollution in the ocean, especially Asian sea. This data will also be used to



develop numerical models to predict MPs in the global ocean. I hope many collaborators in Asian countries will join the monitoring program and share these valuable data.

#### **4.08.P-Mo387 Development of a Semi-automatic Software to Identify Microplastics from Imaging Data by Micro-Fourier-transform Infrared Spectroscopy**

*Yutaka Kameda, Chiba Institute of Technology, Japan*

Small microplastics (MPs) smaller than 300  $\mu\text{m}$  can be easily detected by the imaging technique of Micro-Fourier-transform infrared reflectance spectroscopy ( $\mu\text{FTIR}$ ). In my laboratory, Nicolet™ iN™ 10 MX (Thermo Fisher Scientific) is used to analyze microplastics greater than 20  $\mu\text{m}$  in water, sediment, biota, and the atmosphere. OMNIC™ Series Software is also utilized to identify and quantify various polymers from contour diagrams of polymers, which were measured by the FTIR. However, it has been revealed that the "Profiling" feature in the software often misidentifies polymers. Therefore, many scientists have to reidentify each microplastic particle and fiber extracted by the software. This visual inspection always consumes a significant amount of time and effort. To save time and identify microplastics more precisely, a semi-automatic software, YCALOS13, was developed. The software, YCALOS 13 (You Can LOok microplasticS), is a free software based on a macro in Excel. After imaging analysis of microplastic samples by Nicolet™ iN™ 10 MX, the mapping file is converted to CSV format. YCALOS13 can extract only spectra of designed polymers from more than 100,000 spectra data in the mapping file, based on polymer-specific bands, the peak areas, and their shapes, which users can decide in the configuration. It can generate each contour diagram of many target polymers simultaneously after only pushing the start button. Finally, ImageJ software can quantify the number of MPs from the contour diagram of each polymer. We compared the abundances of MPs in river water samples and sea water samples between OMNIC software plus visual inspection and YCALOS13. It was revealed that YCALOS13 could detect concentrations comparable to OMNIC software plus visual inspection. YCALOS13 can be a useful tool to identify various polymers from imaging data from  $\mu\text{FTIR}$ . This poster will also introduce a novel software "YCALOS-DE," which can also classify each MPs fragment and fiber by MPs degradation level "age." YCALOS-DE will be a useful tool to identify environmental MPs more accurately and rapidly without professional knowledge and experience.

#### **4.08.P-Mo388 Occurrences of Microplastics larger than 20 microns in Surface Waters at Tokyo Bay**

*Yutaka Kameda and Emiko Fujita, Chiba Institute of Technology, Japan*

A release of microplastics (MPs) from the terrestrial environment via rivers is considered to be one of the sources of MPs observed in the ocean. Especially, their occurrences in the surface water of river mouths and bays are influenced by their input from their watershed. On the other hand, ecosystems in bays are very important for human activities such as fisheries and sightseeing, as well as habitats for a huge amount of wildlife. However, ecological risk assessment of microplastics is remarkably difficult because of a lack of information about MPs concentrations, polymer compositions, and size distributions.

The objective of this study is to reveal the occurrences of microplastics larger than 20 microns in surface water at Tokyo Bay, which is considered one of the most contaminated bays in Japan. Microplastics greater than 20 microns were analyzed using micro-Fourier-transform infrared spectroscopy (micro-FTIR) according to the method established by Kameda et al. Several hundred liters of water samples were passed through plankton nets with a pore size of 10 microns on ships at 10 sites in Tokyo Bay and river mouths in 2023. Suspended samples were digested by hydroperoxide in a 200 mL tall beaker. Pretreatment by enzyme was also conducted. Following digestion, sodium iodide was added to the beaker for density separation. Supernatants were obtained three times, and the pooled supernatants were passed through a hydrophilic PTFE membrane. Microplastics greater than 20 microns on the membrane were measured using the imaging technique of micro-FTIR. The poster will demonstrate the concentrations, polymer compositions, and size distributions of microplastics in the surface water of Tokyo Bay.

#### **4.08.P-Mo389 Occurrences of Microplastics larger than 20 Microns in Sediments at Tokyo Bay**

*Yutaka Kameda and Emiko Fujita, Chiba Institute of Technology, Japan*

Microplastics are ubiquitous in various environmental media, as indicated by previous reports. However, detailed information on their toxicity to human health and other organisms, concentrations, polymer compositions, and size distributions, especially for fine microplastics smaller than several hundred microns, remains limited. MPs have also been observed in the sediment of rivers, bays, and seas, in addition to surface water. The presence of MPs in sediment can provide crucial insights into the environmental behavior of plastics from terrestrial areas to the ocean.

This study focuses on the occurrences of MPs in the sediment of Tokyo Bay, which is considered one of the most contaminated bays in Japan. Initially, an analytical method for MPs greater than 20 microns was established using micro-Fourier-transform infrared spectroscopy (micro-FTIR). Subsequently, ten sediment samples were collected from Tokyo Bay using an Ekman sampler in 2023. The moisture contents of these sediment samples were measured in our laboratory. The dried sediment samples were used for the analysis of MPs. In the analysis process, the dried samples underwent digestion by the Fenton reaction in a 200 mL tall beaker. Following digestion, sodium iodide was added to the beaker for density separation. Supernatants were obtained three times, and the pooled supernatants were passed through a hydrophilic PTFE membrane. Microplastics greater than 20 microns on the membrane were measured using the imaging technique of micro-FTIR. The poster will demonstrate the concentrations, polymer compositions, and size distributions of microplastics in the sediment of Tokyo Bay.

#### **4.08.P-Mo390 Occurrences of Microplastics Larger than 20 Microns in Surface Waters of the Philippines**

*Emiko Fujita<sup>1</sup>, Apple AJ Langcamon<sup>2</sup>, Maria Kristina Oquinena Paler<sup>2</sup>, Yuka Motohashi<sup>1</sup> and Yutaka Kameda<sup>1</sup>, (1)Chiba Institute of Technology, Japan, (2)University of San Carlos, Philippines*

In preparation for the international treaty on the reduction of plastic pollution in 2025, and potentially following the ratification of such a treaty, countries worldwide may need to create emission inventories for plastic and monitor environmental concentrations. Moreover, it is estimated that Asia is home to rivers that contribute significantly to global plastic emissions into the ocean. However, there is a significant lack of information on the current levels of plastic pollution in Asian countries, especially concerning microplastics.

The aim of this study is to reveal occurrences of microplastics larger than 20 microns in surface waters of rivers, coastal areas, and off the coast of the Philippines. Microplastic samples were collected at various sites along the Butuanon River, which is the most contaminated river in Cebu. Microplastics were also collected in coastal areas in the Philippines. Microplastics off the coast areas were sampled using volunteer ships. The sampling was conducted as follows: more than several hundred liters of water samples were passed through plankton nets with a pore size of 10 microns on ships or at all sampling sites. Microplastics greater than 20 microns were analyzed using micro-Fourier-transform infrared spectroscopy (micro-FTIR) according to the method established by Kameda et al. Suspended samples were digested by hydrogen peroxide in a 200 mL tall beaker. Following digestion, sodium iodide was added to the beaker for density separation. Supernatants were obtained three times, and the pooled supernatants were passed through a hydrophilic PTFE membrane. Microplastics greater than 20 microns on the membrane were measured using the imaging technique of micro-FTIR. This poster will demonstrate the concentrations, polymer compositions, and size distributions of microplastics in the surface water of the Philippines. This will be the first study to reveal occurrences of fine microplastics in the Philippines.

#### **4.08.PC Marine and Coastal Pollution: Detection, Monitoring, Assessment, Regulation, and Management**

##### **4.09 Microfibre Release From Textiles and Subsequent Pollution: Root Causes, Emission Routes, Effects and Mitigation**

###### **4.09.T-01 Unnatural ‘Naturals’: Sources, Pathways, and Impacts of Textile’s Plastic Alternatives**

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Natural textile fibres (e.g., cotton and wool) are widely marketed as biodegradable, greener alternatives to their plastic analogues, which are major source of microplastic pollution worldwide. In 2015, the potential impacts of these fibre types as a ‘missing link to chemical pollution’ was first suggested. In 2019 the entire environmental textile fibre population was first quantified in an environmental science study, reporting a dominance of natural textile fibres in three UK rivers and atmospheric deposition. Eight years after natural fibres were first proposed as a potentially key particulate pollutant, and four years since their dominance in the natural environment was identified, the scientific community has still not assessed the environmental impacts of one of plastic’s most common alternative materials.

This presentation will summarise a body of work that has investigated natural textile fibre pathways to, fate in, and impacts on freshwater environments. This research includes consideration of the role of hand laundry in textile fibre shedding in the Global South. Informed by ethnographic methodologies and a laundry-independent assessment of the propensity of textiles to shed fibres, we find that the way clothes are made is a key factor in global microfibre shedding. We also explore the movement of different textile fibre types through aquatic environments using an Armfield 10m flume, finding that river characteristics influence the distribution of fibres of all types. The preservation of textile fibres in the lake sediment record is considered using a chemical-free method of textile fibre isolation from aquatic sediments. This approach enabled the extraction of natural textile fibres from a 20cm sediment core dating to at least the 1950s. The majority of fibres isolated from this core were natural, not plastic. Finally this work explores the interactions of chemical pollutants with natural and plastic textile fibres (analysis ongoing).

Findings are considered in the context of sustainable fashion narratives, considering the roles of key groups in efforts to address the problem of textile fibre pollution in the Global North and in the Global South. Concern for the environmental impacts of plastic textile fibres are justified, but replacing these fibres with natural alternatives is inappropriate. These alternatives cannot be used to facilitate business as usual consumption.

###### **4.09.T-02 Accumulation of anthropogenically derived microfibers in a coastal food web and responses in representative zooplankton species**

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Anthropogenic microfibers are increasingly detected worldwide, and textiles are now considered to be one of the largest sources of microscopic waste in marine, freshwater, and terrestrial ecosystems. Microfibers are now considered to be one of the more toxic particle shapes, given that they are difficult to excrete and may be more easily translocated within biota. Toxicity in organisms may be caused by physical interactions between the fiber and organism and/or by toxic compounds released from the particles. We investigated the presence of synthetic and anthropogenic microfibers in zooplankton (prey) and gray whales (predator) which are part of a critical coastal food web off the West coast of North America. Additionally, we examined microfiber toxicity (200 microns length, 3 MFs per ml) using two synthetic textiles (nylon, polyester) and two anthropogenic textiles (cotton, hemp) in representative model zooplankton (*Americamysis bahia*). Field samples were collected via light trap, lab experiments were conducted with cultured mysids. All digestion and analysis protocols followed standard approaches (e.g. KOH, FTIR). In lab exposed animals, reactive oxygen species and behavior were measured. Three species of common gray whale prey, *A. tridens*, *H. sculpta*, and *N. rayii*, sampled off the Oregon coast, had 4 microparticles per gram of composited digested tissue on average;  $\frac{3}{4}$  of microparticles were microfibers with the majority being identified as either anthropogenic in origin (e.g. cellulosic, wool) or synthetic (e.g. polyester). In the lab, MFs became lodged in the mysid (*A. bahia*) foregut area after 7 days of exposure. Ingestion levels were similar to what had been observed in wild zooplankton of relatively the same size and phylogenetic group. ROS was elevated, especially at high exposure temperatures, and was highest with hemp. In conclusion, average ingestion in wild zooplankton was relatively low, the majority of these particles were microfibers, and a sizeable portion were not synthetic, but anthropogenically modified (e.g. cotton). This is in alignment with other recent studies. In the lab, both synthetic and naturally-derived particles can induce changes in ROS production and to a lesser extent behavior. By understanding impacts at the base of food webs can we better evaluate potential microfiber impacts on predators and entire ecosystems.

#### **4.09.T-03 Ecotoxicological Effects of Bio-based Microfibres on the Key Soil Detritivore *Eisenia fetida***

*Winnie Courteney-Jones, Richard D. Handy and Richard C. Thompson, School of Biological and Marine Sciences, University of Plymouth, United Kingdom*

Bio-based plastics are sometimes promoted as less environmentally harmful than their petrochemical-based counterparts. Bio-based fibres are widely used in clothing, sanitary products and wet wipes, and their production is projected to increase over the coming decades. Microfibres can accumulate in soil in substantial quantities through the application of biosolid fertilisers, where they can interact with soil biota. The aim of this study was to compare the ecotoxicity of bio-based (viscose and lyocell) and conventional (polyester) microfibres on the key soil ecosystem engineer, *Eisenia fetida*. In the absence of acute toxicity data, experiments were initially conducted to determine the lethal threshold of each material (0 - 10,000 fibres.mL; 72-hour exposure, OECD TG 207 filter paper method), then a second series of experiments using environmentally relevant concentrations (100 mg.kg<sup>-1</sup> = 0.01%) examined sub-lethal effects across a suite of endpoints including growth and reproduction (28-days, soil exposure, OECD TG 222). The dose-response and the lethality estimates indicate that the bio-based polymers cause greater mortality than conventional polyester to *E. fetida* (LC<sub>50</sub>: 50 mg.L<sup>-1</sup> viscose and 132 mg.L<sup>-1</sup> lyocell), while polyester only achieved 30% maximum mortality despite increasing concentrations to 1000 mg.L<sup>-1</sup>. Following 28-day exposure to soil contaminated with 100 mg.kg<sup>-1</sup> fibres, no significant effects on mortality or growth were observed, however adult bioturbation activity varied significantly between timepoints ( $F_{(3,383)} = 34.166$ ,  $p < 0.001$ ) and treatments ( $F_{(3,383)} = 12.365$ ,  $p < 0.001$ ), where activity was higher in the viscose and lyocell treatments than compared to polyester or control groups. The wet weight of juveniles differed significantly between treatments ( $F_{(3,12)} = 7.472$ ,  $p = 0.004$ ) with lower biomass recorded in the viscose than polyester treatments. Results show that bio-based fibres have the potential to elicit greater toxicological responses than conventional polyester fibres which may have implications on individual fitness, population dynamics, and wider ecosystem function. We emphasise the importance of ecological risk assessment for any materials proposed as plastic alternatives/substitutes.

#### **4.09.T-04 There is much more than Fiber Release during Washing: a Life Cycle-based View on Particle and Fiber Release from Polyester Textiles**

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A lot of research has been performed about release of microplastics from polyester textiles, mostly focusing on the release of microplastic fibers (MPF) during washing. However, washing is not the only life-cycle stage where release can occur and MPF are not the only type of material released. The aim of this presentation is to give an overview of a number of studies we performed with polyester textiles. A main conclusion is that a range different types of materials can be released from polyester textiles during all stages of the life cycle: fiber production, washing, abrasion during use and weathering (outdoor use or end of life), both for virgin as well as for recycled polyester. There is a wide variety of different materials released: MPF, fibrils, odd-shaped microplastics and nanoplastics, depending on the release process. While washing mainly releases MPF and a few fibrils formed during the yarn manufacturing, abrasion produces new MPF but also fibrils. Weathering by simulated sunlight is able to produce a high amount of various fiber and particle shapes. A comparison of virgin and recycled polyester fabrics revealed that there are no significant differences between the two in terms of MPF and fibril release during washing and abrasion. Nanoplastics are released both during washing as well as during abrasion. However, a more systematic investigation of the identity of the released nanoplastics revealed that a large fraction of them are actually water-insoluble oligomer particles and

not polymeric materials. Overall, the results presented here indicate that fate and effect studies about microplastics released from fabrics need to consider other particles types, shapes and size classes than just the “standard” MPF.

#### **4.09.P Microfibre Release From Textiles and Subsequent Pollution: Root Causes, Emission Routes, Effects and Mitigation**

**4.09.P-Tu400 Every workout counts? Concentrations of trace elements, anti-microbials and total fluorine in sportswear**  
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Clothing fibres are ubiquitous contaminants having been detected from the Antarctic to the Himalayas and in a wide range of environmental matrices, including human lung tissue. Trace elements and organic compounds, many of which are recognised as hazardous, are used in the manufacture of clothing from production of the textile through to post-treatment of garments. There is a paucity of information on the types and quantities of chemicals that may be present in different types of clothing and hence associated with the fibres released during wear, laundering and ultimately disposal of the garment. This lack of information on the chemical burden of fabrics hampers our understanding of the environmental and health risks of fibres released from synthetic textiles. Sportswear is a rapidly growing category of clothing that comprises circa 20% of all clothing sold globally. As sportswear is intended to be worn for exercise, anti-microbial, stain resistant and sweat repelling chemicals may be added to reduce the need for laundering and to enhance the life of the garment. The aim of this project was to characterise the concentrations of trace elements, antimicrobial compounds and total fluorine as a proxy for organofluorine compounds in sportswear to identify priority chemicals that may be associated with fibres released from synthetic textiles.

Twenty-six new items of sportswear were analysed for trace elements, total fluorine and organic antimicrobial compounds. Antimony, chromium, zinc and silver were the trace elements measured at the highest concentrations in sportswear with the concentrations dependent on the fabric type as well as the additives added to impart functionality. Lead and mercury were not detected and nickel was only in 4 garments. Total fluorine concentrations ranged from 6 to 40 mg/kg and were lower than previously reported values for clothing known to be coated with poly- and perfluoroalkyl substances. Surface contamination from machinery during processing may be a potential additional source of total fluorine in clothing. Three organic anti-microbial compounds were identified and quantified in some of the items; benzotriazole (ND to 18 µg/kg), methylbenzotriazole (ND to 48 µg/kg) and alkyl tri methyl ammonium bromide (ND-20 µg/kg). Our results indicate that fibres released from clothing are likely to have a high chemical burden and that further research is required to determine the associated environmental and human health risks.

#### **4.09.P-Tu401 Chronic effects of microplastics to *Artemia franciscana* in benthic system**

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Worldwide contamination of microplastic is a global issue in environmental field. Especially for marine ecosystem, microplastics gradually settle to bottom that crawling organisms in benthic system can be easily exposed to sunk microplastics contaminants. Since *Artemia franciscana* from juvenile stage crawls on the bottom performing feeding activity, this study applied microplastic exposure to *A. franciscana* under benthic condition. This study assessed the chronic effects of polyethylene terephthalate microplastics with 3 fragments (small, medium, large) and two fibers (short or long) by comparing their sizes and shapes, and *A. franciscana* at juvenile stage was exposed to contaminated seawater with sand on the bottom. Through 4 weeks of exposure, mortality, growth, and swimming activity were examined. As results, microplastics increased mortality and small-sized fragments and short-length PET fibers caused growth inhibition with movement alterations. These results indicated that microplastic contamination under benthic system can affect aquatic living organisms depending on the microplastic size and shape. *Acknowledgement-This research was supported by “Risk assessment to prepare standards for protecting marine ecosystem” of Korea institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (KIMST-20220383).*

#### **4.09.P-Tu402 Fabrication and Analysis of Fibrous Microplastics for Toxicity and Testing Assessments**

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Fibrous microplastics, constituting a substantial portion (30~80%) of marine microplastic pollution, are known to be released during the laundry of clothing. These fibers entangle the digestive tracts of marine organisms, causing issues such as ingestion disorders and eventual death. It is estimated that they can have a profound impact on humans through the food chain, with fiber-type microplastics being considered among the most hazardous in the realm of microplastics. Household laundry serves as a well-known primary source of these fibers. When microplastics enter the human body, they can induce particle toxicity, oxidative stress, inflammation, tissue migration, and accumulation. Thus, effective management of microplastics is deemed essential. In this study, micro-sized polymeric fibers were fabricated by shredding various fiber materials to assess the toxicity of fibrous microplastics, utilizing equipment capable of maintaining fiber morphology while adjusting only the length. This process can precisely control the degree of fiber refinement by adjusting the number of shredding. The resulting fibrous microplastic samples, diverse in lengths and characteristics, will play a crucial role in conducting comprehensive assessments of environmental behavior and toxicity, as well as research on the occurrence and mitigation of microplastic pollution.

#### 4.09.P-Tu403 Long term exposure of *Lumbriculus variegatus* (Oligochaeta) to nylon-6 microfibers

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Microplastics (<5 mm) are a complex group of emerging contaminants. Their origin (e.g., custom made MP for certain applications) can play a crucial role in their distribution and toxicity in biota. MP in cosmetics (such as sunscreen and hair spray) is an important source of primary MPs to the environment. However, they are severely understudied for their effects, making investigating their toxicity to aquatic biota essential.

To address this gap, we investigated the chronic effects of nylon-6 fibers ( $\varnothing = 14\mu\text{m}$ , length = 500 $\mu\text{m}$ ) to the sediment dweller oligochaetes *Lumbriculus variegatus*, a freshwater model species which is present in a wide variety of habitats in most continents.

Experimental vessels (n=3) consisted of natural lake sediment (70g), spiked with the fibers at concentrations of 0.01, 0.1 and 3% of sediment dw and 150mL of artificial freshwater (AFW; 1mM in Ca<sup>2+</sup> and Mg<sup>2+</sup>). Additional controls (n=3) without fibers, were also set. Phosphate buffer was added to the AFW after week 1 to maintain stable pH level. Two timepoints was assessed: after 28 days and after 30 weeks as long term experiment. *Urtica spp.* was added to the sediment as an extra food source (0.5% of sediment dw). Ten worms, pre synchronized for reproduction (14d before), were added per replicate. The tests followed existing guidelines (OECD, 2007) and the endpoints monitored were reproduction (number of worms) and biomass (dry weight).

Results after the 28d of exposure indicated no effect in the reproduction and growth of the worms with any of the concentrations of microfibers used. The results of the ongoing 30 week experiment will help us to determine the potential effects of microplastic fibers on wildlife on a long-term basis. Further, a possible reduction in the burrowing patterns of worms has been tentatively observed and will be quantified by photographing the sediments.

Overall, this study highlights the importance of introducing long term exposures in ecotoxicology tests of not just microplastics, but all types of anthropogenic chemicals. This would enhance the environmental significance of existing data.

#### 4.09.P-Tu404 Unravelling the Aging Effects of Disposable Masks into Water Towards the Release of Micro-/Nanoplastics and Fibers

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Owing to the excessive usage of disposable plastic face masks (DPFMs) throughout COVID-19 pandemic, their discharge and accumulation in the environment has led to severe pollution issues. Once released in the environment, DPFMs can fragmentate and degrade into smaller particles and fibers after the effect of several environmental stresses (e.g., heat, oxidation, UV irradiation, biofouling, mechanical abrasion) that they can possibly undergo, which can possibly circulate and enter water bodies and food chain, with their effects after their intake for both human and living organisms being still a debatable issue. Since natural weathering is a heterogeneous phenomenon, relatively the slow degradation in natural ecosystems can facilitate the generation of a diverse group of particles with several sizes and morphologies, as well as chemical composition, highlighting the challenges that occur from their discharge. In this light, the aim of the present study is to enlighten the insights into the analytical techniques that can be facilitated to investigate the release of micro-/nano-particles and fibers from DPFMs after simulated UV aging conditions, investigating their role as a newly established core for plastics pollution. State-of-the-art analytical techniques were employed to accomplish this goal, facilitating a thorough inspection of the particles likely to be released from DPFMs matrices. The findings of this study can improve the understanding of the fate of DPFMs in the environment and enlighten the strategies towards plastic waste management.

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#### 4.09.P-Tu405 Simulated Degradation of Polyester Fibres Released from Laundry and with Different Manufacturing Steps

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The pollution of the environment by plastics has been extensively studied during the last two decades. The laundering of synthetic textiles is one of the main sources of microplastics (MPs) in environmental compartments, such as wastewater, sludge and the ocean. The wide use of polyester (PES) in the textile industry contributes to its release and persistence since it cannot be easily degraded. The fate of these synthetic microfibers depends on different parameters, but their photodegradation

due to sunlight exposure is considered one of the most common and effective. Recreating this type of degradation in the lab can be time-consuming and, thus, accelerated protocols may be applied to speed up the degradation process. The release and the degradation of PES fibres before and after washing was studied, using an accelerated hydrolytic degradation protocol proposed by Sarno et al (2021). Three different types of polyester textiles were used: undyed polyester (U-PES), dyed polyester (D-PES), a polyester commercial garment (G-PES). Fibre preparation before degradation was done using three different methods: (1) fibres were manually cut from not washed U-PES and D-PES fabrics, (2) D-PES fabrics were washed under domestic washing conditions (40°C, non-bio detergent, 1200 spin) and the fibres were collected from the drain pipe by a 1µm pore PP filter; (3) G-PES was also washed and the fibres collected as above. Four sampling times were chosen in order to observe the degradation progress. Analysis of all the fibres before and after the degradation was performed by Scanning Electron Microscopy (SEM), micro- FTIR (uFTIR), Pyrolysis- Gas Chromatography- Mass Spectrometry (Py-GC/MS) and High Pressure Liquid Chromatography- High Resolution Mass Spectrometry (HPLC-HRMS). Degradation of the samples was evident, with color and fibre mass loss increasing throughout the procedure. According to the SEM results, the textile type and the washing process did not affect the morphology of the degradation pattern. Py-GC-MS analyses showed differences in the pyrolyzates of the different types of fibres tested. To the best of our knowledge, this is the first study that simulates degradation in fibres collected after washing, determines the effect of laundering on fibre degradation, and compares two PES fabrics that have been processed and used in distinct ways.

#### **4.09.P-Tu406 Who wore it better? Identifying extractables and leachates from textile microfibres**

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Synthetic and semi-synthetic microplastic fibres arising from textiles are extremely abundant within the built environment. Moreover, their proliferation within household dust suggests that inhalation of fibres may be one of the most prevalent sources of human exposure. As opposed to microplastics generated from food contact items (e.g., plastic containers), textiles can have a multitude of chemical additives, with varying degrees of inherent toxicity, such as flame retardants, optical brighteners, and water repellents. Identification of leachates from these microplastics is of high importance in terms of risk assessment and regulation. In this study microplastic fibres generated from clothing were leached to determine the chemicals present in several clothing types. These were three synthetic shirts comprising of 100% recycled polyethylene terephthalate (PET) (blue), 100% PET (blue), 100% polyamide (PA) (black) and one shirt comprising 100% cotton (black). Polymer composition was verified using ATR-FTIR. To identify the chemicals present in each shirt, fabric was cut into ~1cm x 0.5cm irregular pieces and 1g of each fabric was sonicated in 100% DCM (1:10 w/v) in glass vials for 1h at ambient temperature (RT). Thereafter, extracts were analysed with an Agilent GCMSD. To identify aqueous leachates, microfibres were generated using a cryogenic mill. Thereafter, microfibres (0.2 g) were leached in filtered MilliQ H<sub>2</sub>O (1:10 w/v) for 14d at RT on an orbital shaker in the dark. After 14d, nontarget analysis of the extracts was carried out using X500R Quadrupole Time-of-Flight (QTOF) MS (Sciex). Spectral deconvolution and identification of features was carried out using the NIST17 database (GC) or using MS-Dial, MS-Finder and the R package MSCleanr (LC). An MS-Dial MSP spectral database was used for initial searching, whilst a custom-built database using the NORMAN suspect exchange list, in addition to inbuilt MS-Finder databases was used for in-silico searches.

Unexpectedly, when leached in DCM, the PET shirt contained the most features with 555 tentatively identified chemicals, followed by cotton (466), PA (289) and rPET (250). All four clothing items contained chemicals listed on the NORMAN suspect list, and chemicals identified as Substances of Potential Concern. In terms of aqueous leachates, Cotton contained the most identified features (211) followed by PA (185), rPET (112), and PET (112). This poster will present and compare these results.

#### **4.09.P-Tu407 Textile Features Affecting Fibre Release: A Critical Analysis of Empirical Findings and Evidence**

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Emissions of textile fibres into the environment are recognized as a potential threat to humans, wildlife, and ecosystems. This emerging contaminant is thought to affect human health when inhaled or ingested. In wildlife, they are reported to cause inflammatory processes, reduce reproduction and growth, and increase mortality rates. While research into environmental contamination and health issues in humans and wildlife is increasing, the understanding of how emissions occur and the impact of manufacturing features on fibre release is still limited.

Several authors have studied the influence of textile parameters (e.g. fibre chemical composition and length, twist and type of yarn, fabric's construction and density, method used for sewing clothes) on fibre release, yet, most publications fail to demonstrate clear evidence in their findings. A critical review of this evidence is necessary so advancements can be made to mitigate emissions.

In this research, a systematic method was used for finding the literature regarding microfibre release during domestic washing. A search was conducted on 18/04/2023 on Web of Science and Pro-quest databases, from 2010 to 2023. Results were compared and duplicates removed. Only peer-reviewed articles published in scholarly journals and in English were considered. Abstracts were read and potentially relevant papers, selected. Relevant publications were read in full. References from all selected papers were screened and relevant publications were included in this analysis.

46 publications were initially selected. While many publications perceived textile characteristics as having an impact on fibre emissions, such features were not tested in the described experiments, so papers were excluded. Studies were analysed based on their research aims, ability to control variables that could confound results, use of procedural blanks and controls, and use of suitable statistical methods. 70% of publications failed to demonstrate evidence of their findings.

In conclusion, although research about microfibres released into the environment grows at astonishing rates, there are few studies focused on understanding the mechanisms behind this phenomenon. Many features are perceived as having an impact on fibre shedding, but few studies succeeded in demonstrating the evidence of their findings. Our analysis indicates that the quality of studies must be improved so the root causes of microfibre shedding are identified, and mitigation can occur.

#### **4.09.P-Tu408 Quantification and Characterisation of Microplastic Release from Different Aquaculture Nets Towards Emission Reduction**

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The fisheries and aquaculture industries have become increasingly dependent upon large amounts of plastic infrastructure and equipment, which can contribute to the microplastic (MP) emissions to the marine environment. Particularly, net cleaning procedures can cause damage and abrasion of nets, potentially leading to synthetic particles from biocidal coatings and/or fibres being released. This study aims to assess MP release from different nets used in aquaculture and identify easily implementable MP emissions reduction measures using specific combinations of polymers and coatings. A laboratory experiment simulating abrasion damage from *in-situ* net cleaning was performed to quantify and compare MP release from a range of different netting materials, including new and used versions of (Nylon, HDPE, Dyneema, ENTEX) net samples, as well as two coating types used to extend the life of the nets (standard and premium). Nylon net samples from above the sea surface and different depths were also assessed. Abrasion tests were conducted using a Buraschi abrasion simulator system. Triplicate samples of each net and net/coating combination were subjected to abrasion followed by washing and filtration on 500 and 10 µm filters to isolate the ‘large MP’ and ‘small MP’ fractions. The collected material was then quantified and physicochemically characterised using a combination of gravimetric measurements, microscope imaging and chemical fingerprinting by pyrolysis GC-MS. The total MP load was determined for each sample and comparisons between the different net and net/coating combinations made. The results indicate that nylon nets release higher quantities of MP compared to HDPE, Dyneema and Entex. Used nylon net samples from above the water surface and at different depths below the water surface exhibited very different particle release masses, suggesting UV exposure and environmental weathering strongly influences the potential for MP release. While the tested two coatings had no effect on the quantity of MP released from Dyneema nets, being comparable to the uncoated net, a significantly higher mass of MP was released from the coated nylon nets, especially with the premium coating. The outcomes of this study indicate that the aquaculture industry can select specific combinations of net materials/polymers and, coatings that can minimise MP release to the marine environment throughout the usable lifetime of the infrastructure.

#### **4.09.P-Tu409 LIFE CASCADE Project: Removal of Microplastics and Other Pollutants from Textile Wastewater**

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The emission of some emerging contaminants such as microplastics (MPs) and other micropollutants by the textile sector has drawn considerable attention of the scientific community at global level. Some of these substances were used in many industrial sectors for decades, and therefore are ubiquitous in the environment. MPs represent one of the addressed problems, whose origin is largely connected to the textile production cycle and deterioration of textile products. Another important category is that of poly and per-fluorinated substances (PFAS) are among the most persistent micropollutants and among the most studied by researchers due to their impact on the environment and human health. Developing methods for detecting and sustainably removing these micropollutants is important, since existing wastewater treatment plants are not specifically designed for removing these substances. The EU LIFE CASCADE project (EU Call LIFE-2022-SAP-ENV) aims at developing analytical procedures and wastewater treatment technologies meant to detect and remove two very critical categories of micro and emerging contaminants for the textile sector: MPs and PFAS. The project, coordinated by Centro Tessile Serico Sostenibile (CTSS), involves research laboratories of different universities and organizations (Politecnico di Milano, Università degli Studi dell’Insubria, Università degli Studi di Brescia, AquaSoil, Acquedotto Industriale, Biochimie, CITEVE, COMO ACQUA, De Nora, Lariana Depur, ZDHC) with world-class expertise in the management of pollutants in wastewater (WW). The output of the project includes three layers: standardized and multi-lab validated analytical protocols to detect and quantify PFAS and MP contaminants in heterogeneous textile WW; a modular set of WW treatment units to be installed at factory and municipal treatment plant levels; an orchestrating methodology meant to support the design and implementation of the best combination of treatment modules according to the textile district configuration and WW characteristics. Besides, the exposure and effects of PFAS and MPs on aquatic ecosystem in “natural” conditions and after

specific treatments will be elucidated. The project demonstration solutions will be installed and tested in the Como Textile District and lab-scale experiments will also be carried out in cooperation with the Portugal textile district. The poster will illustrate the main features and activities of this four-year project.

#### **4.09.P-Tu410 Influence of the microfiber catcher design on the capture efficiency of microplastic fibers generated during the washing process**

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Fibrous microplastics refer to plastic particles under 5 millimeters in length, and have a length-to-diameter ratio exceeding 3. Particularly, fibrous microplastics generated during the clothing laundering process are emerging as a significant contributor to environmental pollution. They are released into the ocean via laundry wastewater and emitted into the atmosphere by remaining on the clothing surface. Upon discharge into marine environments and the atmosphere, these fibrous microplastics accumulate within organisms, through respiration and ingestion, leading to increased disease incidence and mortality rates due to physical harm and chemical toxicity.

Efforts have been made to mitigate microplastic discharge from household washing machines, introducing devices such as laundry bags and laundry balls to capture microplastics within the drum. These devices effectively capture microplastics from both washing machine wastewater and fabric surfaces, thereby reducing their release into the atmosphere and aquatic systems.

Therefore, this study aims to develop microfiber catcher and investigate how their design affects the efficiency in capturing microfibers. The study considers factors like the number of air meshes, the number and design of the external layer on the efficacy of microfiber capture. The ultimate objective is to develop an optimized disposable fibrous microplastic trap design to aid in resolving environmental pollution caused by microplastics.

#### **4.10.P Progress Into Monitoring and Assessing Risks of Antimicrobials and Antimicrobial Resistance in the Environment**

##### **4.10.P-We367 Key Environmental Drivers of Antimicrobial Resistance**

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Antimicrobial resistance is a global crisis with over 4.95million associated deaths in 2019 alone. Currently there is a lack of knowledge in what causes its prevalence, particularly as it is becoming clear that the acquisition of AMR is more complex and multifaceted than the misuse of antibiotics.

Our research is focused on the characterisation and correlation of biological, physical, and chemical environmental variables to identify key drivers of AMR. In addition, with no monitoring strategy in place we explore the potential of using different toxicity indices.

By analysis sediment and water samples near to chemical water treatment plants, for geochemical properties; physicochemical chemical organic and inorganic pollutants, as well as carrying out microbiome and metagenomic analysis. In addition, we isolated a model eukaryotic organism; *Acanthamoeba sp.* to extract and compare varying levels of resistance and any changes in the microbiome as a result of geochemical changes. Resistance was determined both through susceptibility testing and metagenomic analysis.

We demonstrated that *Acanthamoeba* harbours different bacteria species and can act as a vector for AMR. Further to this, we demonstrate that the need for biological monitoring in AMR detection is a costly process that is difficult to implement on a routine basis. By correlating existing environmental monitoring processes, e.g. of metals and physicochemical properties, and their associated toxicity indices, we can potentially gain an insight to the prevalence of AMR, meaning a cost effective mitigation strategy can be implemented.

##### **4.10.P-We368 Evaluation of the SELECT Assay as a Method to Facilitate Risk Assessment for Antimicrobial Resistance**

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Due to the extensive use of antimicrobials throughout the human, veterinary and agricultural sectors, antimicrobial resistance has become one of the greatest concerns to human health across the world. Though selection for antimicrobial resistance (AMR) has been proven to occur at environmental concentrations of antimicrobials, no standardized experimental method has been designed to determine this. This study aims to evaluate the suitability of the 'SElection End points in Communities of bacTeria' (SELECT) method developed by Murray et al. (2020) by: exploring variability in results across test laboratories, different test matrices; and comparing the results of the SELECT assay to other testing approaches such as a Colony Forming Units (CFUs) approach or qPCR. CFUs, qPCR and SELECT assays were carried out to determine the effects of the fifteen antimicrobials (azithromycin, cefotaxime, chloramphenicol, ciprofloxacin, clarithromycin, enrofloxacin, erythromycin,



gentamicin, trimethoprim, metronidazole, sulfamethoxazole, sulfadiazine, lincomycin, tetracycline, and tylosin) using wastewater samples obtained from five plants. The SELECT assay showed a small variation between the No Observed Effect Concentrations (NOECs). However, none of the antimicrobials had the same NOEC concentration for all five WWTPs. For the eight compounds studied by Murray et al. (2020), at least one of the WWTPs presented the same NOEC concentration reported within the paper. The CFUs approach showed no statistically significant differences between the control and the antibiotic treated plates for all WWTPs. Utilizing a qPCR method, only two compounds (azithromycin and ciprofloxacin) appeared to cause a significant increase in the prevalence of the *int1* resistance gene in at least one of the WWTPs. When the SELECT method was applied the results indicate a small variation between WWTPs and agree with those determined by Murray et al. (2020), showing the transferability of the assay. Given that no statistically significant difference was found when using CFUs, the optical density readings, taken during the SELECT method, appear to be suitable for assessing the effects of antimicrobials on community growth. Furthermore, apart from azithromycin and ciprofloxacin, the SELECT assay appeared to be protective for resistance selection. Overall, the data suggest that the SELECT method is robust a potentially useful tool for use in the environmental risk assessment of antimicrobials.

#### **4.10.P-We369 Occurrences of antibiotics in wastewater and runoff of pharmaceutical production sites and their impact on environmental water quality**

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Antibiotic resistance is increasingly jeopardising the effectiveness of prevention and medical treatment of an increasing number of infectious diseases and is causing a high number of premature deaths worldwide. By now, it is widely recognised that one important contributing factor is the release of production wastewater of antibiotics from the pharmaceutical industry into the environment. Evidently, tackling such point sources through appropriate treatment of production wastewater would be a decisive step towards achieving a substantial reduction in antibiotic pollution and consequently the reduction of occurrences of resistant pathogens. The here presented pilot study addresses the overall feasibility of implementing maximum permitted API concentrations in production wastewater and how to verify compliance. Wastewater from 13 production sites from Europe, India and China has been investigated. The antibiotics tested so far include roxithromycin, ciprofloxacin, moxifloxacin, amoxicillin, cefaclor and levofloxacin. In addition, wherever possible, complementary investigations were carried out in surface waters in the surrounding environment connected to the production sites.

So far, 18 different antibiotics have been detected in concentrations above the respective LOQ, some of them repeatedly and at several sampling locations. Four production sites have shown antibiotic API concentrations exceeding the PNEC - in either the wastewater samples or in the affected environmental samples. These include maximum concentrations ranging from 1 µg/L (tiamulin) up to 500 µg/l (azithromycin). Overall, in the total number of measurements of environmental water samples, more than 50 percent of the antibiotic findings exceeded the PNEC as an ecotoxicologically relevant threshold value, while for other antibiotics detected in the samples such a reliable, scientifically derived effect threshold value was not available at the time.

The results of our pilot study quantify and confirm that wastewater from pharmaceutical production sites is a major contributor to high concentrations of antibiotic APIs in the environment, and hence potential AMR formation. At the same time, this study shows that wastewater treatment genuinely represents an effective opportunity to reduce antibiotic emissions from pharmaceutical production into the environment and thus also to reduce the development of AMR.

#### **4.10.P-We370 Occurrence and Seasonal-Spatial Variation of Emerging Chemicals and Antibiotic Resistance Genes in a Wastewater-Effluent-Dominated Stream**

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Emerging contaminants (ECs), particularly antibiotics and antibiotic resistance genes (ARGs), have been detected in nearly all surface water bodies. This presents a significant and pertinent challenge, as these contaminants have the potential to foster the development of novel resistances within water and surrounding ecosystems, thereby exacerbating the issue of antibiotic resistance. The prevalence and seasonal variations of these pollutants in surface water bodies, such as rivers affected by wastewater treatment systems (WWTPs) discharge, is crucial for assessing and quantifying the associated impact.

In the frame of the Life-BIODAPH20 project, 4 seasonal monitoring campaigns of the Onyar River (Girona, Spain) were conducted during autumn, winter, spring and summer. 52 ECs and 7 ARGs were analysed from water samples collected upstream, at 4 different points downstream and from the secondary WWTP effluent. ECs were detected with an UHPLC-HRMS instrument and ARGs were detected with high-capacity quantitative PCR.

30 out of the 52 monitored ECs were detected in the monitored water samples, ranging from 0.5 to 34923 ng/L. Median concentrations of ECs were higher at the WWTP effluents than up- and downstream. Sucralose and Caffeine were detected at

the highest concentration in the WWTP effluents. Most of ECs detected in the WWTP effluent disappeared 4 km downstream with an average attenuation of  $79 \pm 4\%$ . ARGs were detected at high concentrations in the WWTP effluent and steadily decreased throughout the river (average attenuation= 96%). Int1 and Sul1 were the most abundant ARGs throughout the 4 seasons, followed by tetM, qnrS, blaOXA, blaCTX and blaTEM. A seasonal pattern could be observed between Spring and Autumn.

These findings suggest that in-stream attenuation of ECs, including ARGs through processes such as biodegradation, photodegradation or sorption, represents the most plausible mechanisms for reducing these pollutants. Nevertheless, this data also shows that the studied WWTP has an impact on the river ecosystem that cannot be counterbalanced by natural attenuation processes.

#### **4.10.P-We371 Resistome Profile of the Baltic Benthic Ecosystem**

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The release of nutrients, antibiotics, and pollutants into the Baltic Sea ecosystem through various sources, e.g., wastewater discharge and industrial activities, are the major factors contributing to the introduction and spread of antimicrobial resistance in the area. These impacted areas serve as relevant reservoirs of pathogenic bacteria and antimicrobial resistance genes (ARGs) that pose severe public health threats. Hence, environmental monitoring of antimicrobial resistance throughout the Baltic Sea is warranted. It is also necessary to understand the link between its resistance profile and associated genes against various environmental parameters to aid in identifying potentially high-risk environments and prevent resistance from spreading to low-exposure habitats. In this study, we re-analyzed previously published metagenomic data from benthic sediment samples collected from 59 monitoring stations in the Baltic Sea. We characterized the antibiotic resistome profile of benthic ecosystems and investigated its spatial variability between eight regions spanning 1,145 km across the Baltic Sea. We report the presence and potential dissemination of important ARG types in each region, with concerning observations of multidrug resistance being the most abundant and prevalent type for all stations. We also report the influence of physicochemical gradients, i.e., salinity, temperature, water depth, dissolved oxygen, total carbon, and total nitrogen availability, on the diversity and composition of the resistome and its associated genes. The microbial structure and distribution of resistance and associated genes mainly differed between regions along the spatial distance and physicochemical gradients of the Baltic Sea. This observation implies that future environmental changes in dissolved oxygen, salinity, and temperature may influence the resistome profile of these benthic habitats. Our findings indicate that the Baltic Sea resistome is stocked with resistance genes and represents concerning ARG types that require further monitoring. Utilizing metagenomics tools and combining analyses of resistance gene abundance and its association with other genes and microbial communities, we provide a valuable approach for monitoring antimicrobial resistance in the Baltic Sea environment and assessing their potential dispersal and possible risks to other health sectors.

#### **4.10.P-We372 Grazing on freshwater biofilm influences *Xenopus laevis* larvae microbiota and antimicrobial resistance dynamics in the environment**

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Antimicrobial resistance (AMR) is an emerging concern and considered as one of the biggest threats to public health by 2050, thus being subject of intensive research into this field. It has been largely demonstrated in laboratory exposure that AMR is enhanced by various classes and concentrations of antibiotics. In freshwater ecosystems, antibiotics are rejected by Waste Water Treatment Plant (WWTP) and can impact the microorganisms constituting river biofilms, disturbing their role in primary production, at the basis of the aquatic trophic chain. Knowledge of dynamics of AMR in environment and wildlife as potential hotspots or mitigators are missing. In the present study, the influence of biofilm grazing has been investigated on the dynamics of AMR in two microbial compartments of aquatic ecosystems: the grazed biofilm and the gut microbiota of a model grazer (*Xenopus laevis*). *Xenopus laevis* larvae were fed during 12 days with two different freshwater biofilms from two different sources near (B) or far (A) from a WWTP discharge. The influence of these two types of biofilms was investigated in terms of dynamics of the intestinal microbiota diversity of these organisms, as well as presence of antibiotic resistance genes (ARGs) by MiSeq sequencing and High-Throughput qPCR approach. After 12 days, the microbial composition of grazed biofilms was significantly influenced by the inoculum composition compared to control (without biofilm inoculation). Significant changes in relative abundances of ARGs were also observed. *Xenopus* microbiota were significantly influenced with 12 and 26 changing OTUs abundance in larvae who grazed on Biofilm A and B respectively and a significant change in ARGs relative abundance have been observed with more abundance of *ampC/blaDHA* and *aac-iVa* in larvae grazing on biofilm B. These findings may play a role in: 1) dynamics of antibiotic resistance dissemination in environment and the gut microbiota and 2) the physiological response of the animal. This study paves the way to further research on biofilm microbiota and ARGs dynamics under more realistic conditions to better constrain the mechanisms driving antibiotic resistance fate in aquatic ecosystems.

#### **4.10.P-We373 Political demands to produce antibiotics more environmentally sustainable**

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The supply of antibiotics - whether in human or veterinary medicine - is of outstanding importance to all modern societies. At the same time, industrial production of antibiotics can lead to development and spread of resistant microorganisms through contaminated wastewater. The intention of our work is to extract the main political demands for improving the situation at antibiotic production sites based on practical experiences.

Therefore, the Germany's largest health insurance provider, AOK, has launched a pilot study on sustainable antibiotic supply in collaboration with IWW Water Centre and the German Environment Agency. The contracts with the suppliers of selected antibiotics allowed independent wastewater inspections at the production sites and the obligation to given standards for antibiotic emission. The inspections at ten different productions sites in Asia and Europe included a review of the wastewater treatment technologies used, a joint inspection of the production facility and sampling of production wastewater at the end of the treatment chain. Their measurements of 20 antibiotics show high concentrations in the wastewater and adjacent rivers (see also abstract by Karges et al.). These alarming results pose a danger for the local population and risk for worldwide spread of microbial resistance.

The study shows also positive effects: the knowledge about wastewater treatment increases and improvements were achieved at some production sites. It clearly illustrates mitigation of environmental impact is possible. However, more far-reaching changes than individual supplier contracts are needed to implement such measures globally. The EU could play a pioneering role here. Four main political demands in the field of tension between the three dimensions of sustainability (ecological, social and economic) will be addressed: 1. Inclusion of binding environmental criteria in the EU pharmaceutical legislation; 2. Standardised control systems to compliance during authorisation and ongoing production; 3. Knowledge transfer through partner projects, especially in the Asian region; 4. Shortening supply chains through changes to EU procurement law.

#### **4.10.P-We374 The Antimicrobial Resistance Multi-Stakeholder Partnership Platform: An Inclusive And Collaborative Space For Enhancing Global Governance On AMR And One Health**

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Antimicrobials play a crucial role in preventing and treating infections and diseases in humans, animals and plants. Their over-use is the main driver of antimicrobial resistance (AMR). Poor sanitation, inadequate infection prevention and control practices, and a lack of access to clean water facilitate the treatment-resistant microbe spread. As a result, AMR threatens global health, food safety, food security and economic prosperity, as well as planetary biodiversity and ecosystems.

AMR is a global health and development threat and requires a greater coordination, communication, political leadership, interdisciplinary collaboration in a truly One Health spirit to deliver on the Global Action Plan, National Action Plans on AMR and, ultimately, the 2030 Agenda for Sustainable Development.

The 2016 Political Declaration of the High-level Meeting of the United Nations General Assembly (UNGA) on AMR was a milestone for promoting an ad hoc inter-agency coordination group (IACG), which produced a report 'No time to wait: Securing the future from drug-resistant infections'. The report recommended the establishment of three global governance structure on AMR along with the One Health Global Leaders' Group on AMR (GLG), the Independent Panel on Evidence for Action against AMR (IPEA) and the AMR Multi-Stakeholder Partnership Platform. The latter is a Quadripartite (FAO, UNEP, WHO, WOA) collaboration on AMR and One Health launched in November 2022 by the Quadripartite.

The AMR Multi-Stakeholder Partnership Platform is a global, inclusive and collaborative platform which aims to catalyse a global movement for action against antimicrobial resistance (AMR) by fostering cooperation between a diverse range of stakeholders at all levels across the One Health spectrum.

On 15-16 November 2023, just before the World AMR Awareness Week, the Inaugural Plenary Assembly of the Platform took place in person and online at FAO headquarters. More than 130 member delegates from different countries and stakeholder groups (governments, financial institutions, civil society organizations, academic and research organizations, private sector) meet for the first time, while presenting their proposals for joint Action Groups, discussing Platform's collective governance and priorities for the future, such as the UNGA High-Level Meeting on AMR next Sept. 2024, that will be a historic opportunity to renew political momentum and commitment for antibiotic resistance.

#### **4.10.P-We375 Are On-Site Sewage Facilities Contributing to the Spread of Antimicrobial Resistance?**

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Wastewater is a major reservoir of antimicrobial resistance (AMR) contaminants, both chemicals and antibiotic resistance genes (ARGs). While research often focuses on municipal wastewater treatment plants, on-site sewage facilities (OSSFs) are widely overlooked regarding the occurrence, fate and transport of antimicrobial contaminants. In Sweden, 15% of the households is using OSSFs. As the OSSF effluent can be discharged via soil infiltration to groundwater, an important source for drinking water, water quality assessment is needed.

This study aims to investigate the dissemination of antimicrobial contaminants from OSSFs to groundwater environments, and the related risks of AMR development.

The studied OSSF system serves 300 population equivalents, and samples were collected at the inlet and outlet of the septic tank, and after treatment with aerated ponds over different seasons. Groundwater samples were also obtained beneath infiltration sites and downstream, with upstream samples as reference. Samples were analyzed for antimicrobial chemicals, sewage markers, ARGs, mobile genetic elements (MGEs) and integrons using (SPE)-LC-MS/MS and high throughput qPCR.

Compared to the nearby municipal treatment plant, antimicrobial chemicals were less frequently found and at lower concentrations in the OSSF, attributed to the absence of connections to hospitals or manufacturing industries. Preliminary results show higher relative abundance of specific ARGs associated with aminoglycosides, multidrugs, phenicols, quinolones, tetracyclines and vancomycin resistance, as well as class 1 integrons and MGEs, in the effluent wastewater compared to the influent. ARGs related to aminoglycosides and tetracyclines are additionally found in downstream groundwater but are absent in the upstream samples. Even at non-detectable concentrations of chemicals, microbial selection for AMR can occur, allowing for the proliferation and spread of resistance genes. We will further evaluate removal efficiencies, seasonal correlations between antimicrobial chemicals and ARGs, and the relationship between ARGs and MGEs/integrons, shedding light on the mobility of resistance genes. Risk assessments using predicted no-effect concentrations for quantified antimicrobial chemicals will also be conducted.

Our initial findings suggest that OSSFs may contribute to AMR development and dissemination, highlighting the importance of monitoring these systems as potential AMR sources in the future.

#### **4.10.P-We376 Case study to examine environmentally relevant amoxicillin concentrations in production wastewater - before and after wastewater treatment improvements**

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Antimicrobial resistance endangers the effective prevention and medical treatment of an ever-increasing number of infections and leads to a high number of premature deaths worldwide. Tackling point sources of antibiotic releases into the environment such as the discharge of wastewater from pharmaceutical production through appropriate treatment would be a decisive step towards significantly reducing antibiotic pollution and its consequences.

This is where our pilot study comes in and deals with the feasibility of implementing the maximum permissible concentrations in production wastewater and verifying compliance. Since September 2021, we inspected the wastewater from production facilities whose antibiotics are also marketed in Europe. The companies concerned had committed to comply with a Predicted No Effect Concentration for certain target antibiotics in the production wastewater. In cases where the limits were exceeded, manufacturers were instructed to improve their treatment processes. These improvements were then verified by means of repeated wastewater analyses.

Decisive for achieving an effective opportunity to reduce antibiotic emissions into the environment is ensuring that wastewater treatment is very much tailored to the APIs under consideration, including testing for such APIs. This pilot study led to a growing awareness among producers of the environmental and human health impacts of antibiotic releases. Survey results prompted changes, intensified monitoring and strengthened mitigation measures tailored to the specific situation of manufacturing facilities.

The example presented illustrates the increased benefit of an adapted water treatment system with regard to antibiotic contamination from production wastewater. Wastewater from an inspected site was analysed for amoxicillin concentration. During the initial sampling, wastewater treatment at this site consisted of a tank with a pH of 7-8 to break up ring structures. An amoxicillin concentration of 10 µg/L was detected in the production wastewater, which corresponds to 40 times the threshold value of 0.25 µg/L. As a result, the treatment was adapted by choosing a larger tank, an extended retention time and an optimised pH setting. Amoxicillin could no longer be detected in a follow-up analysis.

#### **4.10.P-We377 Plastics as a Potential Vector for Spread of Antimicrobial Resistance and Pathogens From Wastewater Discharge in the Marine Environment – the PlastiSpread Project**

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Antimicrobial resistance (AMR) and marine plastic pollution are both major environmental and global health concerns. However, there is limited knowledge concerning the occurrence of pathogens and antibiotic resistant bacteria (ARB) in wastewater (WW) effluents from urban areas and in the coastal marine environments into which they are discharged. Furthermore, the potential role of plastics as a vector for the transmission of pathogens, ARB, and antibiotic resistant genes (ARG) throughout the marine environment is poorly understood. The PlastiSpread project studies the role of the microbial community in biofilms associated with marine plastics, and how they are influenced by variations in urban WW discharges. Furthermore, the potential role of marine (micro)plastic litter to act as a reservoir and vector for the development and spread of AMR and pathogenic bacteria in marine environments and into the human food chain is evaluated.

By combining field- and lab-scale plastic model systems, the influence of WW effluent composition and polymer type (PE, PS and PVC), on the bacterial community composition in plastic-associated biofilms will be investigated in relation to other materials (wood, glass). The occurrence of potential pathogens, ARBs and ARGs will also be studied. The laboratory experiments will utilise microplastic forms of each material (300-500 µm) and will be performed both in Norway and Greece. As Greece has among the highest consumption of antibiotics in Europe, such complementary studies will allow investigation into how and to what degree the antibiotic consumption will influence the occurrence of ARB and ARGs in local effluent WW. The field model system will contain plates (10 x 5 cm) of each selected material, which will be placed at exposed and non-exposed sites in the Trondheim Fjord, Norway. Marine bivalve molluscs will be used as indicators for exploring effects on marine organisms present at the same sites. Laboratory experiments will be performed to determine the process of horizontal gene transfer of ARGs from WW to susceptible plastic associated biofilm bacteria.

The knowledge obtained in the PlastiSpread project will have a long-term impact on WW legislation and requirements for how the WW treatment plants design their treatment processes. The project will increase our understanding of marine plastic litter as a vector for pathogens and ARB in the marine environment.

#### **4.10.P-We378 Occurrence of Antibiotic Resistant Bacteria (ARB) and Antibiotic Resistance Genes (ARG) in Water from the Middle Tagus River: Seasonal Variability**

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The Tagus River is the longest river in Spain and around 7 million people live along its course, with 80% of them doing it in its middle section. Unfortunately, overpopulation and related wastewater treatment plants, many different industries, and agricultural and livestock activities are also concentrated in this central area.

The presence of ARB and antibiotic resistance genes ARG in natural ecosystems as rivers, is associated with different anthropogenic activities being the agriculture, the pharmaceutical industry, and the wastewater treatment plant the main contributors for these contaminants.

Data related to the presence of ARB and ARG in Tagus River are very scarce, and that is why we set out to carry out the monitoring of these contaminants in the middle Tagus River basin.

Samples of water were collected from 19 sites that had been selected attending to the closeness with livestock and agricultural farms, pharmaceutical industries, and wastewater treatment plants. Four samplings were carried out seasonally during 2022 and 2023. The presence in the water of bacteria resistant to the antibiotics ampicillin, doxycycline, sulfamethoxazole, and ciprofloxacin was quantified by filtering through 0.45 µm filter membranes and subsequent incubation of the filters on Tryptone Soy Agar (TSA) plates containing the corresponding antibiotics. For ARG analysis quantitative PCR (q-PCR) reactions were carried out. It has been analyzed genes that code for resistance to tetracyclines (*tetA*, *tetB*); sulfonamides (*sul1*, *sul2*); fluoroquinolones (*qnrB*, *qnrS*, *qnrA*); β-lactamase from gram-negative bacteria (*blaTEM*); next-generation carbapenem antibiotics (*blaKPC*); vancomycin (*vanA*); colistin (*mcr-1*) and methicillin (*mecA*), as well as the presence of the integron responsible for the accumulation and dissemination of cassettes containing genes for resistance to cephalosporins, carbapenems and quinolones (*int1*).

Results from this study have displayed statistically significant differences in the content of ARB and ARG for the samples of water taken both from different seasons and different sites and between the antibiotics tested. ARB concentration was significantly higher in the summer water samples, what has been related to the weather conditions and the lower river flow both increasing the antibiotic concentration in the water. In addition, a significant increase in the ARG content has been observed in samples of water taken from sites where anthropogenic activity was higher.

#### **4.10.P-We379 Ecosystem-Level Assessment of The Fate and Effects of Benzalkonium Chloride in In-Lake Mesocosms**

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Benzalkonium Chloride mixtures (BACs, or ADBACs), are some of the most common quaternary ammonium compounds used as active ingredients in sanitizing and disinfecting products, including those recommended for preventing the spread of COVID-19 and other viruses. As a result, their use and presence in the environment have increased over the past few years. Their ecosystem-level effects, however, are still poorly understood, particularly in the lower trophic levels which might be more sensitive to their antimicrobial nature. This study presents the results of an 80-day, in-lake mesocosm study aimed at assessing the fate and ecological-level effects of BAC mixtures. Eight ~5,000L open-bottom enclosures were installed in a boreal lake at the IISD-Experimental Lakes Area (Ontario, Canada). Three enclosures were untreated controls while the remaining 5 represented treatments with 20, 112, 632, 3,557, and 20,000 ng/L of a BAC mixture centered around the C12 and C14 homologs. To simulate a continuous inflow, like what would be expected in the real world, the enclosures were re-spiked weekly to maintain the target exposures. The water-column dissipation half-life of the BAC mixture was estimated at ~5 days, with shorter-chain homologs remaining in the water column longer than longer-chain ones. Sediment concentrations increased throughout the study with preferential accumulation of longer-chain BACs. Treatment effects were clear in the lower-trophic levels with periphyton (measured as chlorophyll) showing concentration-dependent decreases with a NOEC at the nominal 632 treatment level and an almost 100% inhibition at the highest treatment. Ecosystem metabolism (assessed through dissolved oxygen data) was also impacted at the highest treatment level. Analysis of the bacterial resistome in the upper few cm of sediment suggests BAC exposure increases 1) the richness (number of resistance genes detected) and 2) the concentrations of genes, both in a concentration-dependent manner. This was evident for the BAC resistance genes and the genetic elements that promote the presence and dissemination of other resistance (e.g., antibiotic) genes. Maximum measured concentrations for BACs in the environment have been reported at ~2,000 ng/L, a value above those where effects start being apparent in this study, indicating that current levels of BACs in the environment may pose a risk in the higher centiles of the exposure distribution.

#### **4.10.P-We380 "Azole Pesticides and Their Role in Antimicrobial Resistance: Case for *Aspergillus fumigatus*"**

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AMR is global threat, and the environment role, including the climate change, is still under investigation. Recently, fungal pathogens and their resistance attracted more attention because of serious concern. Among the fungi, *Aspergillus fumigatus* (*A. fumigatus*), which natural habitat is soil, is responsible for invasive disease and chronic infections in immunocompromised patients.

For the aspergillosis treatment, one of the larger used substance of the pharmaceuticals is the azole molecule. The azoles are also used as pesticides and biocides to prevent and control plant diseases. Additionally, azoles are widely used in animals and in household and personal care products such as soaps and shampoo.

In recent years, increase of resistant *A. fumigatus* has been observed raising high concern since growing evidences would suggest that *A. fumigatus* acquires resistance when exposed to azoles in the environment. Indeed, azole resistant *A. fumigatus* isolates have been found in patients not previously treated with azole-based pharmaceuticals.

The close correlation between environment and human urges for an action since the environment could be a reservoir of resistant fungi due to the constant environmental exposure to the azoles. Furthermore, the climate change could exacerbate this problem as increasing global temperatures can promote the pathogens' survival.

The aim of this study was to investigate if *A. fumigatus* is resistant to a selected panel of sixteen azole fungicides. To do so, the wild type *A. fumigatus* and the most common mutant TR<sub>34</sub>/L98H were exposed to the azole substances and the Minimal Inhibitory Concentration (MIC) was determined. MIC test provides information concerning the susceptibility or resistance of the fungi to the antifungal agent.

Furthermore, soils samples were analyzed for the presence of the *A. fumigatus* and MIC analysis upon exposure to the sixteen azole fungicides were tested. In order to understand the mutations that could cause the resistance, Sanger sequencing for colonies isolated from the soil samples were performed.

In conclusions, the MIC analysis to determine the susceptibility or resistance should be taken into the chemical risk management to prevent and mitigate the spreading of fungal resistance to humans.

## 4.10.PC Progress Into Monitoring and Assessing Risks of Antimicrobials and Antimicrobial Resistance in the Environment

### 4.11 Protecting Innovation in Plant Protection: Low-risk Pesticides, Precision Applications, and Considerations on Risk Assessment

#### 4.11.T-01 Nano-enabled strategies to enhance biological nitrogen fixation

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Synthetic nitrogen fertilizers are widely used to supplement soil fertility, despite their low delivery and use efficiency. This contributes significantly to greenhouse gas emissions, ammonia volatilization and loss of reactive nitrogen into the water from land. Reducing nitrogen fertilizer application is critical to mitigating food insecurity and global warming. Enhanced biological nitrogen fixation (BNF) in crops offers an opportunity to reduce nitrogen fertilizer use and increase yields. The results from two studies investigating nanoscale micronutrients (NiO, MoS<sub>2</sub>) important to nitrogen fixation will be presented. A full life cycle study was conducted to compare soil-applied NiO nanoparticles (n-NiO), NiO bulk (b-NiO), and NiSO<sub>4</sub> at 10–200 mg kg<sup>-1</sup> on soybean. n-NiO at 50 mg kg<sup>-1</sup> significantly promoted seed yield, fatty acid and starch contents by 39, 28 and 19%, respectively. The increased yield and nutrition were attributed beneficial impacts on photosynthesis, mineral homeostasis, phytohormones, and nitrogen metabolism. Single particle inductively coupled plasma mass spectrometry (sp-ICP-MS) confirmed that the majority of the Ni in seeds is in ionic form, with only 28–34% as n-NiO. Separately, low dose (10 mg/kg) MoS<sub>2</sub> NPs enhanced soybean BNF and grain yield by 30%, compared with conventional molybdate fertilizer. MoS<sub>2</sub> NPs can more sustainably release Mo, which is then used for nitrogenase synthesis and molybdenum-based enzymes that subsequently enhance BNF. Sulfur is also released sustainably and incorporated into biomolecule synthesis, particularly in thiol-containing antioxidants that protected the nodules from reactive oxygen species damage and delay aging, maintaining BNF function for a longer term. These studies demonstrate that nanotechnology can sustainably enhance BNF and crop yield and to combat global food insecurity.

#### 4.11.T-02 *Bacillus* strains as biological tools in agriculture - Lets take a look at the soil microbiome and farmers practice

**Sebastian Hartmann-Wittulsky**, Bayer AG

Treatments with living *Bacillus* bacteria, such as *Bacillus subtilis* or *Bacillus amyloliquefaciens*, are widely used in agriculture as part of sustainable farming strategies. These bacteria can be found in plant protection products (PPPs), biofertilizers or biostimulants. They benefit crops in several ways when present in soil leading to an increased productivity in agriculture. As biocontrol agents they are antagonistic against plant pathogens by releasing antimicrobial compounds and trigger defense mechanisms via Induced Systemic Resistance (ISR). Research on biofertilizers and biostimulants has shown that *Bacillus* strains can help plants to tolerate abiotic stress and improve nutrient uptake either directly or indirectly. Furthermore, such bacteria are well known to produce certain phytohormones to modulate plant root growth making them for instance more susceptible to colonization by microbes and increased nutrient uptake. However, is an agricultural soil treatment with living *Bacillus* bacteria impacting existing soil microbiomes short-term or even long-term? What is the value of such treatments for farmers and the environment? It is well known that commercially available *Bacillus* strain do have certain capacities to influence soil microbiomes, e.g. due to the release of antimicrobial compounds, resource competition, biofilm formation, impact on soil processes, and the modification of root exudates. With this presentation we are going to summaries two recent microbiome studies performed in different crops treated with living *Bacillus* bacteria. In both studies microbiomes were analyzed using next generation sequencing (NGS) and high throughput sequencing (HTS) technology coupled to bioinformatics. To connect this to the bigger picture, we will further shed some light on the direct benefits to farmers when using living microbes in their cropping system and how this can lead to a reduced need for synthetic PPPs. Examples of disease management strategies in certain crops including treatments with *Bacillus amyloliquefaciens* strain QST713 will be presented. Such biological PPPs are a tool to support sustainable farming approaches by reducing old synthetic PPPs, but are not sufficient to fully replace classical chemicals to ensure high profitable cropping.

#### 4.11.T-03 Precision application of pesticides in agriculture: role and contribution of the European Precision Application Task Force

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Agricultural practices in Europe are seeing a significant evolution towards precision technologies, evolution triggered by both the fast technical developments in digital sciences and farming equipment. The Farm to Fork and Biodiversity strategies of the European Green Deal and the objectives set for all components of sustainable food systems are accelerating this technology evolution. Precision applications are part of this evolution, and stakeholders agree on the urgent need to link the new technologies in use in crop protection to the regulatory context. The European Precision Application Task Force (EUPAF) was created in June 2023, with the objective to provide a scientific platform to gather expertise in precision applications and risk mitigation measures, and engage a dialog between experts in the development of precision application equipment, farmers, regulatory scientists and risk assessors from both the public and private research sector. EUPAF counts 77 experts from 40

organizations and 14 countries. The Task Force is working on an inventory of current equipment, spraying and/or application practice and technique in use, for arable and permanent crops and use this inventory to define classes or categories of use reduction, and risk reduction, enabled by the equipment and practice.

On the basis of the inventory, the experts are developing a harmonized description of the use categories to use into regulatory dossiers for plant protection products. The description should include the aim of the treatment, a precise description of the area treated and proposal on how to best express in the GAP table information on the type of equipment, any necessary recommendation regarding the performance and efficacy of the treatment, additional agronomic advice for e.g. in terms of resistance management, and the category of use reduction / risk reduction.

For each use category, the description enables to evaluate to what extent the use will lead to significant changes in a particular area of the risk assessment, through a significant modification of the exposure and provide first recommendations as to how the exposure assessment can be adapted. The talk will present the general approach and the current results of the EUPAF.

#### **4.11.T-04 Modelling the Reduction Effect of Spot Applications on Pesticide Runoff Losses from Fields with a PRZM-VFSMOD Coupling and a 2D Probabilistic Framework**

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Field “spot treatments” of agrochemicals through smart agricultural solutions can significantly reduce agrochemical application in the field. This localized chemical application with high resolution (small spot footprint, <1x1 m) can reduce off-field drift and surface runoff impacts to adjacent water bodies.

While quantification of drift reduction is advancing, very limited work exists for the quantification of potential pesticide runoff reduction from this smart agricultural practice in the regulatory context.

For the case of runoff reduction, small application footprint technologies mean that a very small part of the field becomes a source of pesticide runoff during a subsequent rainfall-runoff event. Particularly for crops with dense surface cover (e.g. cereals), the rest of the untreated field can act as a sink for dissolved and adsorbed pesticides before exiting the field. Part of the difficulty in quantifying the potential reduction of pesticide runoff stems from the pseudo-random pattern of spot applications in the field during each crop season and across years during long-term regulatory assessments.

A new 2D mechanistic-probabilistic modeling framework, SPOTRUN, was developed that considers i) sources: upslope pesticide runoff generation from the spot treatments around the field; ii) sinks: infiltration, sedimentation and pesticide trapping on downslope segments (after spots) of the field with vegetation, iii) variable conditions of the field vegetation during the crop phenology and the effects on runoff and sedimentation, iv) size of spots and percentage of field area treated. v) long-term exposure calculations (PEC<sub>sw</sub>). In a first step, a pseudo-random application pattern on the field is generated based on spot size and treated area fraction. The field is then divided into strips in slope direction with the same width as the treated spots. These strips are subsequently simulated with a coupled PRZM-VFSMOD modelling system, which can deal with complex sequences of treated spots (sources, simulated with PRZM) and untreated areas (sinks, simulated with VFSMOD) along the flow path. For each rainfall event in the scenario (e.g. FOCUS<sub>sw</sub>), the edge-of-the-field runoff hydrographs and associated sediment and pesticide loads are summed up and fed into a TOXSWA metamodel. Different realizations of the pseudo-random application allow to construct a probability distribution of PEC<sub>sw</sub> for each event and thus identify realistic worst-case application patterns.

#### **4.11.P Protecting Innovation in Plant Protection: Low-risk Pesticides, Precision Applications, and Considerations on Risk Assessment**

##### **4.11.P-Tu411 How to Improve the Risk Assessment for Low-Risk Pesticides**

*Gertie Arts Mrs., PhD and Héloïse Thouément, Environmental Risk Assessment, Wageningen University & Research (WUR), Netherlands*

Increasing the number of low-risk pesticides is considered as an important step forward to a more sustainable agriculture. Active substances regulated under Regulation (EC) No 1107/2009 are only categorized as low-risk if they meet specific additional criteria. Currently no synthetic chemical, but only naturally occurring active substances (covered here by the general term “biopesticides”) are classified as low-risk. Here, the results of a literature review will be presented on the risk assessment for biopesticides covered by the pesticide Regulation (EC) No 1107/2009. This review includes the following topics: which guidance documents apply, what problems are encountered with the authorization of these substances, which research questions are still open and what can be learned from other regulations to achieve a more targeted risk assessment. The substances considered here include microorganisms (which contain organisms such as bacteria, algae, protozoa, viruses, fungi, baculoviruses); naturally occurring substances including botanicals (plant extracts) and e.g. salt/minerals, blood, chitin; semiochemicals (pheromones i.e. substances that are excreted by an organism and influence the behavior of other organisms). Regulation (EC) No 1107/2009 has recently been adapted for micro-organisms (Part B of the regulation) by including focused



uniform principles and data requirements for microbial pesticides. However, such an approach has not been implemented for other biopesticides, which are regulated under Part A of the same regulation together with the synthetic chemical pesticides. The lack of specific risk assessment approaches for biopesticides hinders their approval. Moreover, biopesticides registered as low-risk have a clear position in Integrated Pest Management (IPM) approaches as introduced by the 2009 Sustainable Use Directive. However, despite the potential function of low-risk pesticides mainly as fungicides and insecticides, low-risk pesticides are currently mostly allowed for use in specialty crops and viticulture, with only 20 % of them being applicable in cereals. To improve the risk assessment of low-risk pesticides, we recommend to develop focused risk assessment approaches for biopesticides starting with a problem formulation.

#### **4.11.P-Tu412 Addressing Current Challenges in the Risk Assessment of Low-Risk Pesticides**

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Potential low-risk pesticides (LRPs) of natural origin are gaining popularity, particularly in response to the European Green Deal. Yet, their data requirements within the authorisation process in the European Union (EU) are largely similar to those of synthetic pesticides, which may hinder the market entry of potential LRPs. Tailored guidance has already been developed for some groups of potential LRPs and the EU-funded project RATION (grant agreement N° 101084163) set out to significantly contribute to improving the risk assessment procedures for potential LRPs of natural origin. Within RATION, as a very first step an overview was prepared on pertinent regulatory and guidance documents related to the authorisation of microorganisms, plant extracts, pheromones and other semiochemicals, as well as ds-RNA and microbiome solutions as plant protection products.

The publicly available “EU Pesticide Database” was analysed with regards to the number of approved and non-approved active substances of various groups, i.e., microorganisms, semiochemicals, plant extracts, substances from non-plant sources, inorganics, and synthetic organic molecules. The first encountered problem here was that the database barely differentiates between these categories. In addition, the whole terminology surrounding LRPs of natural origin is suffering from ambiguity. It was observed that from all these groups, some compounds were approved as low risk active substances and some were not. The date of approval influenced this status as the criteria for ‘low risk’ were not in place when all of the currently approved active substances were evaluated. A more detailed on-going analysis aims to identify the challenges in their data requirements and support the development of further guidance and an improved risk assessment strategy. The comparison of the regulatory frameworks for potential LRPs in the EU and the United States (US) was another aspect of the present study, which was addressed at a general level and detailed by two case study examples, one plant extract and one microorganism.

The results will be used for identifying the differences in approval by product and length in time. This has important implications for the decisions of companies to invest into these products. Research has shown that the uncertainty with respect to the time length as well as the related costs has a substantial effect on the investment decision.

#### **4.11.P-Tu413 Crop protection by RNA interference – a horizon scanning of approaches to inform risk assessment**

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RNA interference (RNAi)-based crop protection aims to inhibit the expression of specific essential genes in target organisms by uptake of double-stranded (ds)RNA and subsequent degradation of target gene mRNA. Due to their biological nature and the relative instability of RNA molecules, RNAi applications are assumed to be less harmful for the environment compared to conventional plant protection products (PPPs). While RNAi-based plant protection was initially limited to genetically modified crops producing dsRNA, the development of exogenous technologies like RNAi sprays has increased in recent years. Due to its assumed beneficial properties, RNAi technology receives increasing attention in the development of PPPs.

A literature research was performed using established literature databases as well as patent databases to identify the current state of RNAi developments and applications in plant protection. RNAi applications were categorized based on the following criteria: 1) treated crop species, 2) target pest species, 3) target gene function, 4) type of application and 5) biomanufacturing readiness level.

More than 180 publications have been evaluated. A great variety across treated crops, targeted pest species and functions as well as application types was found. The current state of development of these applications mainly includes the identification of suitable target functions in pest species as well as testing the functionality of these applications in pest-host systems in laboratory studies. In total, RNAi applications for more than 30 different pest species were identified. Most pest species belonged to the insect orders Lepidoptera, Hemiptera and Coleoptera, besides which also applications targeting fungi and viruses were found. The main targeted biological functions in insects were cell cycle/cell integrity and development whereas in fungi, the cell cycle/cell integrity and pathogenesis mechanisms were targeted mostly.

Concluding, RNAi applications were identified to be an upcoming topic in plant protection. Because of the high number of targeted pest species as well as the variety of application types, which differed from common plant protection approaches in some extent, RNAi applications have an increasing relevance for environmental risk assessment. The present literature review is intended as a horizon scanning project to identify current needs and aims for the development of a suitable risk assessment strategy for RNAi based crop protection.

#### **4.11.P-Tu414 A new class of active substances: dsRNA-based pesticides for Plant Protection – a regulatory perspective**

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RNA interference (RNAi) is a natural process with important defence and regulatory functions in animals, plants and fungi. Mode of action is a gene silencing mechanism capable of reducing or switching-off the expression of individual genes. Sprayable dsRNA-based plant protection products are in the pipeline aiming at different targets such as flea beetles in oil seed rape, fusarium diseases in barley, or weed control to overcome resistant weeds. It is expected that these products, which should be much more specific regarding target organisms than conventional pesticides and less persistent in the environment, will be used in the field in the near future. In that case, RNAi constitutes a new mode of action that is not completely covered by the current authorisation process, that mainly focuses on chemical compounds. While the characteristics of dsRNA as active ingredient might ease the assessment of some risk areas, for others there is a need for adaptations of existing or the development of new risk assessment tools. The risk assessment might also be challenged by new formulations aiming for the prevention of rapid degradation of dsRNA after product application. Therefore, these type of products generate new demands on the approval as active substance laid down in the regulation (EC) No 1107/2009 and also in the data requirements under regulation (EU) No 283/2013. The presentation will shed light on the authorisation process in the EU and where further research and data are needed. Furthermore, it will highlight important aspects regarding scientific challenges, considerations and the current status for risk assessment and risk management for this new type of plant protection product.

#### **4.11.P-Tu416 Toxicity of the Bioherbicide Pelargonic Acid Varies for Different Primary Producers**

**Judith Epping and Paul van den Brink, Wageningen University & Research (WUR), Netherlands**

Biopesticides are considered a possible replacement for currently used synthetic pesticides. Since they are based on naturally occurring substances such as plant extracts, they are frequently considered less harmful to the environment and, therefore, also called low risk pesticides. One example is pelargonic acid, a plant-derived broadband herbicide. Aquatic primary producers, including algae, and rooted and floating macrophytes, essential in maintaining freshwater ecosystem functions, are non-target organisms to this bioherbicide. So far, reported toxicity values of aquatic primary producers vary substantially. The objective of this research is to shine light into the toxicity of analytical grade pelargonic acid to standard and non-standard test species of rooted (*Myriophyllum spicatum*, *Elodea nuttallii*) and floating macrophytes (*Lemna minor*, *Salvinia minima* (to be conducted)) and algae (to be conducted). Five concentrations in the range of 9.4 mg L<sup>-1</sup> to 150 mg L<sup>-1</sup> were tested under optimal growth conditions, closely following OECD guidelines 201 “Freshwater algae growth inhibition test”, 221 “*Lemna* sp. growth inhibition test” and 239 “*Myriophyllum spicatum* toxicity test”. Growth variables including dry weight (all), shoot growth (rooted macrophytes) and coverage (floating), as well as pH and oxygen concentrations were evaluated. Preliminary results show substantial differences in species sensitivity with *L. minor* being most sensitive with significant coverage reduction at 9.4 mg L<sup>-1</sup>. Both rooted macrophytes showed higher tolerance with shoot growth and oxygen concentrations significantly declining at concentrations of 37.5 mg L<sup>-1</sup> and higher. Dry weight was a less sensitive endpoint than coverage or shoot growth. Experiments will be continued in the coming months with two algae species (to be determined) and *Salvinia minima* as a non-standard floating macrophyte. These results are essential to understand environmental implications of the usage of plant-derived pesticides and will lead to a more precise risk assessment in the future.

#### **4.11.P-Tu417 Evaluation of The Environmental Benefit and Impact of Nitrapyrin**

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In light of the recent EU policy developments towards reducing the use and risk from pesticides it is identified that there is a need to also examine the environmental benefits and potential challenges of other chemicals used in agriculture such as fertilizers. Nitrapyrin, a widely used nitrification inhibitor, plays a crucial role in enhancing nitrogen use efficiency while improving crop yield.

Nitrapyrin can reduce nitrogen losses through leaching and denitrification. By retaining bioavailable nutrients like nitrogen within the agricultural system, nitrapyrin supports crop yield while reducing the need for additional nitrogen fertilizers, improving economic gains for farmers. Additionally, the reduced nitrogen runoff helps protect aquatic ecosystems, preventing eutrophication and maintaining water quality.

Nitrapyrin has a history of safe use since its introduction in the 1970s and has an EPA Green Chemistry Award for its environmental and economic benefits. Under EU Regulations nitrapyrin is registered as a fertilizer product, which requires a minimal environmental dataset. However, to fully evaluate the potential environmental benefit and potential impact from the use of this product, a literature review (with no chronological limits or restrictions in research areas) has been conducted based on EFSA guidance requirements for plant protection products. Key areas identified in the search covered the impact on soil microbial composition, nitrogen transformation including greenhouse gas production and potential exposure and effects to terrestrial non-target organisms.

The focus of this poster presentation is to provide a thorough overview of the key findings on the research on nitrapyrin, relating to its environmental impacts while demonstrating that its use as a fertilizer in agriculture is beneficial towards maintaining high yield sustainable, agricultural ecosystems.

#### **4.11.P-Tu418 Biomarkers for Phosphine Resistance in *Tribolium castaneum*: Based on Transcriptomics and Machine Learning approach for Rapid Identification**

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Methyl bromide, a substance once used globally to control stored-grain pests during quarantine processes, was regulated due to its classification as an ozone-depleting substance under the Montreal Protocol. As a result, phosphine (PH<sub>3</sub>) has emerged as a primary alternative fumigant for stored-grain pest control. However, continuous phosphine usage has led to the development of resistant pest populations, causing significant economic losses. Identifying phosphine-resistant pests is crucial to mitigate these losses. The traditional method, FAO test, is time-consuming, and an alternative mutation analysis targeting the dihydrolipoamide dehydrogenase (DLD) gene can only identify strong phosphine resistance at levels 50-100 times higher. In our preliminary research, we used adult *Aus10* (PH<sub>3</sub>-Susceptible, S) and *Aus07* (PH<sub>3</sub>-Resistant, R) strains of *Tribolium castaneum* for transcriptomics, resulting in the identification of 16 genes with significant expression differences. Subsequently, we quantified their mRNA expression levels using RT-qPCR during the life cycle stages, including adult, pupa, prepupa, lateral larva, and early larva stages, according to the 2<sup>-ddCt</sup> method. In addition, machine learning was employed to select three genes according to fold-changes and *p*-values from RT-qPCR data, effectively distinguishing between the two strains at all stages. Validation experiments were conducted with RT-qPCR. Finally, an assessment into the expression levels of these three genes was conducted using the wild strains collected from several grain warehouses in South Korea to confirm their susceptibility and resistance to PH<sub>3</sub> in consistent with FAO test. This study proposes a novel and rapid method for identifying phosphine resistance in various life cycle stages of *T. castaneum*.

#### **4.11.P-Tu419 Environmental Evaluation of the Biostimulant *Methylobacterium symbioticum***

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In light of EU policy developments towards reducing the dependency on chemicals and fertilizers in agriculture, biostimulants are recognized to provide a sustainable alternative to improve plant nutrient use efficiency, tolerance to abiotic stress and increasing crop quality and yield. The EU Fertiliser Product Regulation (FPR) has recently been amended to include biostimulants. This allows a transparent assessment of the efficacy and to examine the environmental benefits and potential challenges of their use.

The bacteria species, *Methylobacterium symbioticum* SB23 is a naturally occurring bacteria biostimulant used to increase nutrient efficiency, uptake and availability.

The strain has been assessed according to the FPR to address potential plant pathogenicity and to provide a detailed assessment of the origin and natural occurrence of the *Methylobacterium* genus. To further expand on the environmental benefits and impacts of the genus, a literature review search was conducted in accordance with EFSA's guidance for the submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA, 2011).

The focus of this poster presentation is to provide a thorough overview of the key findings on evaluation of *Methylobacterium symbioticum* relating to its environmental impacts for use as a biostimulant.

#### **4.11.P-Tu420 Challenges of Paraffin Oil in Aquatic Risk Assessment: A Call for Situation-based Higher-Tier Testing**

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Paraffin and other oils are used as insecticides, offering a potential means to reduce the reliance on synthetic organic pesticides in agriculture. Presently, several products containing paraffin oil as an active ingredient are approved in the EU/Netherlands under the current risk assessment framework. However, the complex and varying composition of these type of oils (alkanes from C15 to C30), and their insolubility in water, pose challenges that are not adequately addressed by the current regulatory risk assessment methods. These challenges include direct and indirect effects of an oil layer on the water surface or dispersed

oil drops for aquatic organisms. As such, a refined approach to the risk assessment of paraffin oil in aquatic systems is needed. Our research seeks to underscore the limitations of current assessment methods and shed light on the intricate exposure and effects of paraffin oil in aquatic environments, advocating for a novel approach to environmental risk assessment.

We identified two distinct exposure situations as relevant for aquatic organisms covering the major route of exposure through drift deposition after a spray application in a nearby field. In the first situation, an oil layer forms on the water's surface, directly impacting organisms that live attached to the water surface and indirectly affecting all organisms in the water column due to the limitation of oxygen exchange with the atmosphere. In the second situation, the oil is dispersed within the water column (e.g. due to wind dispersing the oil layer), primarily affecting organisms that reside in the water column and could come into direct contact with the oil drops, e.g. filter feeders.

Fate models are currently not developed to simulate oil layers. In order to be pragmatic and make a consistent link between exposure and effects in the assessment, we propose to use higher-tier effect testing methods, i.e. mesocosm studies, to assess the effects posed by paraffin oil and other mineral oils to aquatic organisms for both exposure situations. These situations should be assessed through realistic worst-case test setups with regard to the exposure and the composition of the aquatic ecosystem. Our proposal integrates the exposure and effects assessment within two higher-tier test situations and links functional and taxonomic groups to specific exposure routes, in this way addressing the complexity of paraffin oil's behaviour in aquatic ecosystems in a pragmatic approach.

#### **4.11.P-Tu421 Soil Specific Outcomes in the OECD 216 Nitrogen Transformation Test**

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The OECD 216 test guideline investigates the impact of agrochemicals on soil nitrogen transformation, however, it has come under criticism for a perceived lack of . To explore reasons behind this, we conducted an extensive evaluation of 465 previously conducted OECD 216 studies. In doing so, we discover that test outcomes may be dependent on the soil in which the study was conducted. We identified two contrasting outcomes within the control, non-treated samples: the "rise" response, characterized by consistent increases in nitrate concentrations throughout the test period, and the "dip" response, characterized by an initial decline in nitrate concentration between days 0-7, followed by a subsequent net-generation of nitrate between days 7-28. We suggest the lack of consistency in response between control samples from standardized and internationally recognized test guidelines is problematic and concerning. We argue that the dip response undermines the intended functioning of the test system and compromises the ability to draw accurate ecotoxicological inferences from the data. Our hypothesis suggests that the dip response may be a result of conducting the study in low nitrogen content soils. To explore this, we conducted experiments using ammonium sulphate as an immediately available inorganic nitrogen source, replacing the guideline-mandated complex organic nitrogen source: lucerne meal. Our results indicate that the dip response can be alleviated by this substitution, highlighting the influence of nitrogen availability and accessibility. However, not all low nitrogen soils exhibited the dip response, suggesting the involvement of additional unidentified factors. Based on our findings and real-world regulatory examples, we propose that datasets displaying the dip response should not be considered valid OECD 216 studies. In this case, the influence of soil properties on the performance of the test system precludes an accurate assessment of whether observed impacts are solely driven by the test compound or are instead a product of the soil used. To address this issue, we suggest incorporating techniques to account for soil-specific responses into the conduct and interpretation of OECD 216 studies. These proposed amendments aim to enhance the reliability and robustness of the study system, ultimately increasing confidence in the ecotoxicological conclusions derived from OECD 216 datasets.

#### **4.11.P-Tu422 Non-Target Arthropods (NTA): What Influences their Sensitivity to Pesticides in the Field/Landscape?**

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Higher tier NTA studies measure the responses of local communities to the exposure of a plant protection product in highly complex natural systems in contrast to lower tier lab studies, where experiments are conducted under predefined conditions. These natural systems are complex, and NTA communities are shaped by biotic and abiotic factors, which increase the variability in the system. Consequently, several factors could influence the response of the arthropods to the pesticide exposure. Habitat structure and condition (e.g., vegetation cover), abiotic factors (e.g., temperature, humidity), exposure routes and the biology of the arthropods (e.g., habitat preference, feeding strategy) could affect such differences in response.

In our experiments, we see species belonging to the same arthropod families responding differently to the treatment with plant protection products, which cannot be explained by the arthropods' physiology, only. For example, springtail species spending relatively more time within the soil, might be less affected by the overspray of a plant protection product compared to species, which live mostly above soil.

Here, we investigate, if the differences observed in the effect of the application of plant protection products on closely related taxa is correlated with abiotic factors (e.g., temperature, humidity), habitat structure, life history traits (e.g., resource use, dispersal, habitat preference), or the interaction with other arthropods (e.g., prey suppressing one another's abundance through

competition for enemy-free space). Incorporating this additional information about ecology and behavior of the arthropods could lead to a more holistic approach to risk assessment.

#### **4.11.P-Tu423 Bat Activity in Arable Farmland: Assessing Potential Risks from Plant Protection Products**

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Bats are commonly present in farmland, feeding on insects, and may therefore be exposed to residues of Plant Protection Products (PPPs) via dermal contact, inhalation and consumption of prey and contaminated water. A review by the European Food Safety Authority (EFSA) in 2019 found that the potential risk to bats from the use of PPPs in the EU is not covered in the current risk assessment. In order to develop a risk assessment methodology for bats, essential ecological knowledge in how they use the farm landscape is required. The aim of this study, was to explore how bat activity varied with changes in cropping at different growth stages throughout the year.

Six passive bat detectors were mounted at different sites within Cockle Park Farm, a research farm at Newcastle University in NE England. Each detector was fitted with a directional microphone and situated at the edge of a field, with the microphone directed into that field. The cropping at each site differed, including winter wheat, spring barley, peas, permanent grassland, woodland edge (control) and farm buildings. The detectors were left out for 1 week every month from May 2022 to October 2023 (18 months). During this time, the crops grew, were harvested and new (different) crops were sown at each site. The bat echolocation recordings were analysed using the BTOPipeline auto-classifier, with additional manual validation for all calls identified as being from uncommon species and 1% for all calls identified as being from common species. Additionally, potential confounding variables (e.g., temperature, wind, rain) were recorded at the farm meteorological station and used in the data analysis.

Initial analysis indicates up to 10 species of bat present in the summer season, associated with the highest activity levels (number of recordings). Into autumn, bat activity declined as expected with a move to torpor, but also species diversity declined with implications for choosing which to make focal species in a risk assessment context depending on the proposed application timing. Data analysis is still ongoing, but further results and conclusions on the differences in bat activity with cropping and growth stage, as well as in relation to environmental variables, will also be presented.

#### **4.11.P-Tu424 Herbicide prioritization and risk reduction in perennial crops**

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The non-targeted, non-needed, and repeated applications of the same, few herbicides registered in perennial crops had resulted in reduced efficacy, development of herbicide-resistant weed biotypes and increased risk to agroecosystem compartments and human health. Preferential flow and other herbicide leaching mechanisms, runoff, enhanced biodegradation, other herbicide dissipation processes and the increased numbers of herbicide-resistant weeds forced farmers to increase the application doses, and the overall production cost. Moreover, herbicides are among the most frequently detected pollutants in environmental samples, including surface- and ground-water bodies. The disturbances exerted by repeated agricultural practices in perennial crops are a major factor influencing weed biodiversity and population dynamics. Weed density in an agroecosystem and the various soil-climatic conditions and cultivation practices are factors affecting the frequency of herbicide application making the decision support system particularly complicated. In this study, we have created an intelligent herbicide prioritization and application system that will support the decision for the application of herbicides in a targeted, timely manner with great precision, and improved agricultural practice (quantitatively, qualitatively and from an environmental point of view) while the required amounts of herbicides will be reduced, contributing to the Green Deal and Farm-to-Fork objectives and agroecosystem sustainability. The proposed risk reduction scheme is based on the hierarchy of the herbicides registered in perennial crops. Potential low risk herbicides in terms of environmental fate, resistance development and hazard assessment were also included in the priority. Based on herbicide physicochemical properties and their environmental fate, herbicide resistance classification and weed flora biodiversity indices a matrix-based fit-for-purpose scheme was established for perennial crops. The proposed scheme contributes to reduced environmental contamination and delays herbicide-resistance development.

#### **4.11.P-Tu425 Reduction of Pesticide Risk using Optimal Volume Rate Tools and Spray Drift Reduction Techniques**

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During the application of plant protection products (PPP) part of them is lost to the environment and can harm nearby residents, bystanders, flora and fauna. With the aim of reducing risks associated with PPP applications in two important Mediterranean vertical crops, citrus and vineyards, the LIFE PERFECT project “Pesticide Reduction using Friendly and Environmentally Controlled Technologies” (ref. LIFE17/ENV/ES/000205) was carried out focussing in two aspects 1) the use of Optimal Volume Rate Tools (OVRA), CitrusVol for citrus developed by IVIA and Dosaviña UPC for vineyards developed by, based on the adjustment of the spray volume rate to the treatment characteristics (vegetation; orchard; pest or disease:

PPP); and 2) the use of spray drift reducing technologies (SDRT) such as low drift nozzles, air deflectors, together with the calibration and proper adjustment of the sprayers, adapting the spray profile to the target vegetation.

The objective of this study was to determine ground losses and spray drift during PPP applications in citrus and vines, and to evaluate the effect of the optimization of the application, based on the spray volume rate adjustment, fine-tuning of equipment configuration, and use of low drift nozzles.

Results showed that in citrus, while CitrusVol recommended a volume rate 48% lower than that conventionally used, its combination with the SDRT reduced airborne drift by 81% and ground losses by 86%. Considering together all losses in citrus, they accounted for 27% of the PPP applied in the conventional treatment, while this number decreased until 5% in the optimized treatment, which is 81% lower. In the case of vineyards, the optimization in average reduced the volume rate by 27%, airborne drift by 64% and ground losses by 50%. Thus, in this case total losses accounted for 12% of the PPP applied in the conventional treatment, while this number decreased until 6% in the optimized treatment, which is 50% lower

In conclusion, since environmental risks posed by the application of PPP depend directly and proportionally on the amount of PPP used and PPP losses, this work demonstrates the joined effect of 1) volume rate adjustment through the use of developed OVRA tools, 2) use of low drift nozzles, and 3) spray cloud adjustment, on the environmental risk reduction. Therefore, a more sustainable application of PPP is achieved from the environmental point of view.

#### **4.11.P-Tu426 How much an adequate spray volume can contribute to the pesticide use reduction?**

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The optimization of spray application through a proper setting of the already in-use sprayers can be the most effective strategy to comply with the Green Deal strategy requirements. In this sense, the DSS can help farmers to define the adequate spray application rate according to crop type and canopy characteristics. The aim of this work was to quantify the potential PPP reductions achieved in citrus and vineyard, along two growing seasons, thanks to the use of Citrusvol and Dosaviña® DSS, respectively. The canopy characteristics, at the time of applications, were measured and used as inputs for DSS to define (as output) the adequate spray application rate. Then, the DSS-based application rates were compared to those used by farmers (reference), and the PPP use reduction was calculated. Results underline that higher volume were always applied in citrus compared to vineyard. With respect to vineyard case, the PPP reductions achieved were more constant in citrus due to the less pronounced canopy variation throughout the season. In general, the PPP use reduction were higher than 30% in citrus, and 45% in vineyard. These percentages were similar across the two-year considered. In conclusion, the DSS resulted an effective tool to reduce PPP use, and therefore, chemical inputs in agriculture. Thus, DSS can consistently contribute to comply with the Green Deal PPP reduction goal.

#### **4.11.P-Tu427 Precision application of pesticides - Possibilities and challenges from a regulatory perspective**

*Balthasar Smith, Jonas Schartner and Achim Gathmann, Federal Office of Consumer Protection and Food Safety (BVL), Germany*

There is no good reason and no real need for a "business as usual" approach to plant protection. Everyone involved has a special responsibility to support the process towards a more sustainable use of plant protection products in their area. The possibilities for making the use of plant protection products more efficient are better than ever these days. The extent to which plant protection products are used can be completely redefined. However, careful consideration must be given to how risk assessment and risk management interact. The right questions need to be asked. The potentially lower inputs into the environment resulting from the partial area application must be set against an understanding of the exposure pathways, the available data and the uncertainties of the models used. This understanding must also be applied to the risk reduction measures and the consideration of precision application techniques within the framework of toolboxes for combinable measures.

The presentation will illustrate the perspective of a regulator within the framework of the EU-wide harmonized approval procedures for plant protection products in accordance with Regulation (EC) 1107/2009. The focus is on the requirements for risk reduction measures with regard to practicability, effectiveness and controllability.

#### **4.11.P-Tu428 Smart Stewardship in Digital Farming: GIS-Supported Advice for Reducing Groundwater Vulnerability Risks through Optimized Use of Plant Protection Products**

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The rising demand for sustainable and efficient farming practices has led to the development of innovative tools that integrate GIS and digital farming. One such tool is the Groundwater Vulnerability Tool, a Spatial Decision Support System (sDSS) that advises farmers on optimizing herbicide use while minimizing groundwater contamination using Germany as an example.

The sDSS tool builds on the methodologies described in the FOCUS Groundwater guidelines which define the regulatory requirements for assessing the leaching potential of pesticide active substances and their metabolites to groundwater. Leaching

needs to be assessed in nine different pedoclimatic scenarios that combine environmental conditions and farming practices which represent the 80<sup>th</sup> spatial percentile of arable area in the EU. The 80<sup>th</sup> temporal percentile of the predictions is compared to the limit value for active substances in groundwater (0.1 µg/L) to evaluate the leaching risk.

The sDSS assesses groundwater vulnerability and provides farming advice by simulating leaching masses of active substances and metabolites into the soil using the FOCUS PEARL simulation model. This is done based on long-term climate data, soil maps, groundwater levels, and application patterns. The groundwater vulnerability is calculated based on the number of years with leaching masses exceeding a quality threshold. A regression model is derived between precipitation and vulnerability for each representative soil based on the numerical simulations to create a map with spatially explicit application recommendations. Additional features like potentially drained areas and water protection zones can be added as overlays.

A first validation of predicted leaching masses was conducted by comparing them to predictions for the FOCUS GW scenarios. The Hamburg scenario resulted in the highest leaching mass followed by Kremsmuenster and Châteaudun. The results of the spatial simulation are in the same range with an upper bound of leaching mass close to the results of the Hamburg scenario. The sDSS tool provides an overall similar level of protection as required to comply with the EU legislation 1107/2009, but with a higher spatial resolution and regional specific environmental information. Hence, this study exemplifies how digital farming tools can assist achieving localized risk management and thus contribute to sustainable agricultural practices.

#### **4.11.P-Tu429 Precision Application of Plant Protection Products: Risk Assessment Approaches and Potential as Risk Mitigation Measure**

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Agricultural practices in Europe are evolving towards precision technologies like precision application (PA) of plant protection products that allow farmers to apply only the needed amount of the most suitable product at the right time and location. As stakeholders agree on the need to link the new techniques and equipment to the regulatory context, the European Precision Application task Force (EUPAF) was created in June 2023 with the objective to provide a scientific platform to gather knowledge and expertise in PA techniques as well as new risk assessment approaches and risk mitigation measures. This presentation will provide proposals agreed on by the members of EUPAF representing key stakeholders, including experts from the area of plant protection product providers, application technology providers and regulatory risk assessors.

Based on an inventory of current equipment and application practices, PA use categories will be defined and assessed for their potential for product use reduction. For each PA use category, the description allows evaluating to what extent the use would lead to changes in a particular area of the risk assessment (e.g., for operators, bystanders, consumers, non-target organisms) through a modification of the exposure and proposing adapted exposure assessment scenarios to be used in the risk assessment.

A reduction of risk would not be equal for all exposure and risk areas. Specific evaluations are necessary to determine the extent and applicability of the different PA techniques with regards to the different risk assessment and mitigation areas. In a first step, it should be assessed if the use category leads to no/medium/substantial change to the risk assessment assuming current regulatory environment and currently available data as well as future developments accounting for field-specific and application-specific (digital) assessments including control options that allow for documentation of applications. Many PA methods result in non-uniform applications in the treated area and the heterogenous exposure on the field would allow differentiated risk diagnosis and management for the field scale compared to simple worst-case assumptions. A conversion factor for each use category (“if use category j is used, reduce risk by factor k in risk assessment area l”) will be considered for each risk assessment area.

#### **4.11.P-Tu430 Integrating Precision Application into the Regulatory Environmental Risk Assessment Scheme: A Conceptual Approach**

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Precision application is an advanced farming strategy supporting a more efficient and sustainable future for agriculture. There are variety of precision application technologies currently available for the application of Plant Protection Products (PPPs) that, in addition to other agricultural management profits, could reduce the release of chemicals into the environment. However, although these promising methodologies are available and ready to use, their characteristics and benefits are not yet considered for the evaluation and authorization of PPPs under EU Regulation No 1107/2009. Incorporating precision application methodologies into the regulatory framework will help to support the European Commission’s Green Deal objective of achieving a more sustainable agriculture.

The objective of this study is to provide useful and effective information that builds up the knowledge to include these methodologies into the EU environmental risk assessment scheme. For that, a literature search on the application technologies currently available in the market and a review of these technologies used in the regulatory dossier has been conducted. The outcome is a general framework summarizing the potential impact of these technologies on exposure calculations and on the effect assessment on non-target organisms considered within the EU evaluation of PPPs. In addition, a conceptual approach

that describes different options for integrating the precision application methodologies for the calculation of predicted environmental exposure to organisms and its implication on the effect evaluation is provided. Ecological aspects that should be considered when using precision application are also highlighted. Overall, this work shows the needs for the quantitative adaptation of the EU environmental risk assessment scheme to achieve a harmonized regulatory framework

#### **4.11.P-Tu431 Drift Exposure After Patch Application: Modelling Effects on Lemna**

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Patch application, characterized by targeted and localized pesticide treatments, is becoming more common in agriculture and can significantly reduce pesticide loadings to the field. Adopting patch spraying approaches can also reduce off-field drift, surface runoff, and drainage loadings to adjacent water bodies thus reducing off-field pesticide exposure and environmental impact. The present EU risk assessment required for authorization of plant protection products (PPPs) considers a homogeneously sprayed field as the standard scenario, with the above-mentioned entry pathways into adjacent water bodies. Surface water exposure of PPPs applied in patches could be approximated by reducing the exposure from full field treatment by the percentage of treated area in some cases, for example, for exposure *via* drainage or leaching to groundwater. However, this is not the case for exposure *via* drift and runoff as exposure in such cases depends on the distance between the treated area and waterbody.

In this case-study, we have investigated the drift exposure of pesticides in a ditch resulting from patch application by using existing exposure models and scenarios (FOCUS SWASH) to simulate and predict distribution of exposure in space and time. We have compared the pesticide distribution from a patch application using linearly extrapolated Rautmann drift for a single patch and three patches to the measured drift values for a single patch obtained from a drift deposition field trial. The study places particular emphasis on identifying hotspots of exposure where pesticide concentrations are notably elevated, as well as the implications for effects on aquatic organisms. TOXSWA simulations were run for several scenarios, *i.e.*, applications in different months, in order to investigate the plume distribution under different hydrological conditions. The potential implications for the aquatic risk assessment would be investigated using a tiered approach, first a standard Tier 1 assessment would be done by comparing the PEC<sub>max</sub> values followed by more realistic ecological (TKTD) modelling. Also, from simulations of the effects of the concentration profiles of separate segments, a worst-case segment would be selected based on the simulated effect, rather than the exposure profile.

This study aims to provide insights on how an aquatic risk assessment for patch application could be done for regulatory purposes by establishing a link between exposure and effect modelling.

#### **4.11.P-Tu432 New Developments in Spray Drift Modelling for Precision Applications**

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Precision application of plant protection products (PPPs) is receiving a lot of attention as a way forward in pesticide application, since it has the potential to reduce the amount of pesticides applied and to reduce the exposure of different off-field areas and non-target organisms to PPPs. Precision application for crop protection could play a role in the exposure assessment of PPPs in the authorisation process. Basically, variable-rate and spot-spraying applications can be distinguished. In a variable-rate application the whole field is treated while the dose is varied to meet the conditions of each location in the field. In a spot-spraying application for each in-field location the decision is made whether or not a (full dose) spray application is required. Similar to full-field spray applications, with precision applications the downwind deposition of spray drift not only depends on the application technique but also on environmental conditions. In this study we focus on spray drift and its potential reduction for variable-rate and spot-spraying applications in arable crops under different environmental conditions. Recently, a new spray drift model was developed which takes into account the non-uniform dose rates applied at the crop field. Ongoing model development includes the implementation of effects of wind speed, crop height and drift mitigating measures. The model can be used for both variable-rate and spot-spraying applications. Downwind deposits of spray drift are evaluated at off-field evaluation strips, such as adjacent watercourses or non-target terrestrial areas. Spatial resolution of the downwind deposits is adjustable to a desired level by dividing the evaluation strips into imaginary segments of adjustable length. Downwind spray drift deposits depend on the location and distribution of the treated in-field areas and the dose rates applied. Wind direction is the major environmental parameter involved. Variable-rate and spot-spraying applications may lead to drift reduction, partly due to the reduced amount of PPP used. Example computations indicate that drift reductions can be higher or lower than the reduction based on reduced amount of PPP. In the current study, a few test cases are investigated for realistic distributions of wind directions throughout the year. In this way, exposure to spray drift and the potential of precision applications can be assessed on field level under realistic environmental conditions.

#### **4.11.P-Tu433 Environmental Risk Assessment of Different Protection Zones for Non-Target Plants with Precision Application of Herbicides**

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Precision agriculture has emerged as a promising approach to optimize farming practices while minimizing the overall use of



pesticides. Despite the reduction in applied pesticides in the field, it remains unclear how this translates to off-field environmental concentrations and reduced risks to non-target organisms. This study delves into the environmental implications of precision farming by conducting a comparative analysis between conventional pesticide application and mitigation methods and a targeted pre-emergence herbicide spot-treatment. The primary focus lies in evaluating the predicted environmental off-field concentrations, both terrestrial and aquatic, with a comprehensive assessment of associated environmental risks for non-target plants.

Employing a case study framework, we utilized modelling techniques to simulate herbicide spray drift to non-target plants in off-field sections (10m length) along the field in the terrestrial evaluation zone (1.5m – 2.5m) and to the surface water evaluation zone (3m – 4m). Specifically, we assessed the risk associated with a herbicide for which the label requirement for potato crops indicated a DRT90 with 3m buffer zone to protect the surface water organisms. We therefore evaluate worst-case drift scenarios for conventional full-field applications, incorporating various drift-reduction technologies and buffer zones, respectively, in comparison to a pre-emergence spot-treatment case-study.

We present and discuss the results, and give further recommendations for future case studies. This research contributes valuable insights into the environmental risks associated with different pesticide application strategies and in particular with precision agriculture. By highlighting the differential environmental impacts, we can set the environmental risk of precision agriculture in comparison to conventional measures to reduce pesticide usage. Thereby our study is a starting point to highlight the potential and limits for precision agriculture, and sets a unique example in the yet empty field of case studies related to environmental risk of precision agriculture.

#### **4.11.P-Tu434 Consequences Of Reduced Application Of Plant Protection Products On Soil Contamination And Soil Fertility**

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Plant Protection Products (PPPs) are widely used in conventional agriculture to protect crops and to support yields. However, once applied, PPPs can leach into the soil where they can persist for several years. This can lead to chronic exposure of soil organisms that perform important soil functions supporting soil fertility. There is increasing evidence of adverse effects of PPPs on soil communities and their functioning, which can lead to reduced soil fertility including reduced resilience to biotic stress, such as soil-borne diseases. There is a need for a more sustainable approach to agriculture that minimizes the impact on soil organisms.

The PestiRed project aims to reduce the PPP use by 75% and to test alternative measures for weed, pest and disease control, mostly based on agroecological principles. Such innovative measures are tested on 67 farms across Switzerland. For each farm, a control plot is managed conventionally (without PPP reduction) and the impact of management change is monitored over six years.

To investigate the consequences of a reduced PPP use on soil contamination, concentrations of PPP residues will be measured on 32 farms for both innovative and control plots. Soil samples are collected in winter on four years to assess trends over time. PPP residues are measured by liquid chromatography coupled to tandem mass spectrometry using a quantification method recently established for PPP residue monitoring of agricultural soils in Switzerland. The method is highly sensitive (sub-ng/kg range), validated and precise, and allows the multi-residue detection of about 150 active substances.

Standard ecotoxicological tests will be carried out on soil samples from the PestiRed farms to assess the consequences of management change on the fitness of soil invertebrates. In addition, key soil functions indicative for soil fertility, such as nutrient cycling, plant growth promotion and disease suppression will be investigated through field assessments and greenhouse experiments. Finally, microbial and invertebrate communities will be analyzed for the sampled soils. The results of the different experiments will help to establish soil food webs and to better understand the relationships between soil organisms and functions. Overall, the information generated will provide a holistic view of the effects of a reduced PPP use and management change over time on the soil system and the consequences for soil fertility.

#### **4.11.P-Tu435 Developing Landscape Environmental Risk Assessment (ERA) for pesticides under PARC: Conceptualization**

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A project under PARC Task 6.4.4 is developing Landscape ERA, linking regulatory and retrospective assessments to address the overall impact of pesticides on agricultural ecosystems. The landscape consideration is part of the integration proposed by the EFSA PERA proposal and focuses on the spatial and temporal connectivity between pesticide applications and exposure of non-target organisms in agricultural ecosystems. The proposed framework was developed following a set of expert workshops, and is highly flexible, covering generic and real landscape definitions with an implementation that should facilitate the comparison and interconnectivity of different tools. The main element of a landscape framework is the distribution of crop and non-crop areas in a spatial dimension, represented in the proposed framework by the horizontal (XY) plane. Non-target individuals or groups are associated to different crops and other areas, according to their habitat needs and behaviors. Human management includes pesticide application and other agricultural activities, and the landscape structure and the environmental conditions determine the fate of the applied pesticides according to their intrinsic properties. The spatial and temporal concordance determine the exposure of the non-target organisms, which can be modelled at individual, population or community/function/service level depending on the model. The simplest regulatory application is the assessment of aggregate effects from the use of the same pesticide in different crops and the influence of the landscape structure and management on the environmental risks. These assessments support decisions on the identification of priorities, comparative assessments, and mutual recognition. The second step is the integration of the risks associated with all pesticide uses during the season/year. This is essential for quantifying the overall risk of pesticide use, supporting regulatory decisions on sustainable use and integrated pest management, and for comparing the impact of pesticides with other stressors as part of biodiversity protection strategies and policies. The next step under the PARC project is to explore the capacities of this framework in a set of case studies, covering terrestrial and aquatic systems.

#### **4.11.P-Tu436 Developing Landscape Environmental Risk Assessment (ERA) for pesticides under PARC: Selection and Design of Case Studies**

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Under PARC Task 6.4.4 a framework for Landscape ERA was proposed following expert workshops. A landscape framework is characterized by the distribution of crops, non-crop areas, and non-target organisms in a spatial dimension. The landscape structure defines the level of agricultural intensification/diversification, driving pesticide use, and environmental fate. The temporal dimension includes human management, environmental conditions, and population dynamics. The spatial and temporal concordance determine the exposure of non-target organisms as a function of their habitat and behavior. The effects can be modelled at individual, population or community/function/service levels depending on the model. In order to explore the applicability of this conceptual framework, case studies covering terrestrial and aquatic systems has been designed. The case studies are reflecting different agricultural landscape conditions and climates, four covering terrestrial systems and four covering aquatic systems. Three terrestrial case studies (CS) have been built around a common crop, vineyard, exploring

regional, agronomic, and ecological differences: CS1: Farmlands in central Spain, led by UCLM with contribution from ISCI; CS2: Vineyard area in the south of France, led by INRAE and OFB; CS3: Bairrada wine producing region in Portugal, led by UC with contribution from UAVR. The fourth terrestrial case study, CS4: Hope Farm arable farm in UK, led by UKCEH, covers a farm with specific management for biodiversity protection. The aquatic case studies are connected to the EU Water Framework Directive and the equivalent in Switzerland; CS5: Pesticides and WFD in Poland, led by IEP-NRI; CS6 Kleingewässer-Monitoring KgM on small-medium streams in Germany, led by UFZ with contributions from UBA and UKOLD; CS7 Danish stream case study, led by NIVA; and CS8 Aquatic biodiversity status of five agricultural sites in Switzerland, led by EAWAG and the Swiss Centre for Applied Ecotoxicology. The design of the cases considers the needs of regulators, farmers, and different regional stakeholders. Validation is a key component for regulatory use; a classical approach, comparing predictions with measurements would require full knowledge of the impacts of stressors other than pesticides which currently is not available; other approaches, e.g., validation of specific steps, qualification for specific uses, or identification of key elements for regulatory results verification/use are explored.

#### **4.11.P-Tu437 Modelling Aquatic Drift Exposure from a Patch Application**

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Recent technological improvements in pesticide application equipment made it possible for pesticides to be applied precisely to the intended sub-field target areas rather than as a broadcast spray. Surface water exposure of plant protection products applied in patches could be approximated by reducing the exposure from full field treatment by the percentage of treated area in some cases, for example, for exposure *via* drainage or leaching to groundwater. However, this is not the case for exposure *via* drift and runoff as exposure in such cases depends on the distance between the treated area and waterbody.

In this case-study, we have investigated the drift exposure of pesticides in a ditch resulting from patch application by using existing exposure models and scenarios to simulate and predict distribution of exposure in space and time. We have compared the pesticide distribution from a patch application using linearly extrapolated Rautmann drift for a single patch and three patches (1D) to the measured drift values for a single patch obtained from a drift deposition field trial (2D).

TOXSWA simulations were run for several scenarios, *i.e.*, applications in different months, in order to investigate the plume distribution under different hydrological conditions. We have selected the months based on the average residence times in the ditches covering very short to long residence times with the pesticide application happening on the 1<sup>st</sup> of each month. Three different approaches to drift were simulated in TOXSWA: i) drift deposition from a fully sprayed field; ii) linearly extrapolated Rautmann drift values (1D) for a single patch and three patches; and iii) spatially explicit drift from a patch obtained from the drift deposition trial. A generic substance with a  $K_{oc} = 10 \text{ L.kg}^{-1}$  and a long half-life in water ( $DT_{50} = 1000\text{d}$ ) was chosen to investigate the distribution of the substance in water after the drift event based on dynamic hydrological conditions.

The concentration plume resulting from the above approaches were compared and preliminary results have shown that distribution of the concentration plume with time depended on the hydrological conditions (flow velocities) following the application. This study aims to provide an approach to set the exposure *via* drift from patch application into the framework of aquatic risk assessment and that such an exposure assessment is possible using the existing regulatory acceptable approaches and models.

#### **4.11.P-Tu438 Spray Drift Assessment For The Registration Of Plant Protection Products Applied By Drones – Working Within The Existing Regulatory Framework.**

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The use of drones for precision application is fast becoming an alternative for the safe application of Plant Protection Products (PPPs) in the field. The advantage of this technology is a more efficient and safe application compared to traditional methods in areas of difficult access (e.g. steep-slopes, waterlogged soils or rice paddies), precision bait or spot application. The use of this type of precision application would result in a lower environmental impact of pesticides. The existing regulatory framework, Regulation (EC) No 1107/2009, currently lacks the integration of drone technology. Authorities have shown interest in regulation of drone application of PPPs, whilst pointing out the scarcity of reliable data on parameters such as spray drift. Several working groups with members from authorities, industry and research organisations, are collaboratively compiling data to establish use scenarios based on realistic parameters, which could be used for accurate risk assessment, and adaptation of the regulatory framework for drone-applied PPPs. Until now, the most common approach is to consider the use of drones as aerial application. Several studies, however, show that the spray drift values associated with drone use are more comparable with those in terrestrial field application, and lower than those in aerial application.

This poster aims at describing the difficulties of the environmental exposure assessment of spray drift entries of drone-applied PPPs to surface water bodies during the registration process in the EU. Further, it provides example calculations of predicted environmental concentrations in surface water (PEC<sub>sw</sub>) based on spray drift values from literature sources and from within the regulatory framework (aerial and airblast application based on *Rautmann et al.*). The poster proposes an interim tiered approach for a conservative environmental assessment of spray drift of PPPs applied by drones, until regulatory-accepted

drone-specific spray drift values become available. As a first tier, it is suggested to consider worst-case drift rates defined for aerial application. It is further recommended to perform calculations using drift values from literature and/or field studies for drone applications, alongside standard calculations for airblast application. This dual-calculation approach gives the authorities certainty by demonstrating that literature/field study drift values result in lower drift values than those from aerial application.

#### **4.11.P-Tu439 Assessment of Unmanned Aerial Spraying Systems as an Effective and Environmentally Friendly Tool for Citrus Growers**

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Precision agriculture is a key component to a productive agriculture that meets the highest safety expectations for humans and the environment. Unmanned Aerial Spraying Systems (UASS) constitute a promising tool to apply pesticides precisely, reducing carbon footprint and water waste.

The Sustainable Use Directive 2009/128/EC prohibits aerial application of pesticides. Due to the great interest and need for UASS application, many initiatives are generating data to confirm the safety of UASS applications for human health and environment.

This work aimed to design trials to determine whether efficacy and residues levels differ between UASS and ground application in 3 dimensional crops. During protocol development, we determined that working height below 1.5-2 m were needed to avoid excessive drift, and that product application over a tree in main vegetative stage made it difficult to access the innermost parts of the canopy. Preliminary trials were performed to determine if UASS' propellers movement would open the foliage sufficiently to enable a good coverage. The trials were performed with a bait formulation, Spintor® Cebo, which contains an ingredient attractive to the targeted pest, and can be used at very low doses of the active substance Spinosad. Spintor Cebo is designed for patching and banding application, this makes the treatment of the whole area unnecessary and constitutes a significant reduction of environmental impact in comparison to a conventional formulation.

Three trials were carried out in 2022 to compare drone vs. ground application. The objective was to determine the efficacy and residues of Spintor Cebo in mandarins to control *Ceratitis capitata* in the Mediterranean EPPO zone, according to GEP and EPPO standards. Each trial consisted in three treatments: control, ground and drone treatments and four replicates for each treatment. Determination of spinosad residues in fruit were performed by QuEChERS followed by HPLC-ESI-MS/MS.

Results show an efficacy, selectivity and residue levels in the same range between UASS and ground application. These encouraging results indicate that UASS are valid tools for precision agriculture that contributes to meet sustainability goals, while addressing farmers' needs.

#### **4.11.P-Tu440 Review on Pesticide Pollution and Ecological Status of Lake Tana Sub-Basin, Ethiopia**

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The Lake Tana sub-basin in Ethiopia has witnessed substantial changes in its ecological status over recent years, primarily attributed to the increasing prevalence of pesticide pollution due to agricultural intensification. This review aims to reveal the evolving pesticide contamination within the Lake Tana sub-basin and its consequential impact on the structure and functioning of the ecosystem. Drawing upon a wide range of studies and scientific literature, this review explores the types and uses of pesticides and their risks to aquatic biodiversity within the Lake Tana sub-basin.

The first section of the review provides an overview of the historical context of pesticide use in the region (Amhara region), highlighting the factors contributing to the intensification of pesticide pollution. Subsequently, an in-depth analysis of the various types and uses of pesticides detected in Lake Tana, tributary rivers, associated wetlands, and surrounding districts.

In assessing the ecological status of the Lake Tana sub-basin, this review also examines the risks of pesticides on aquatic biodiversity, particularly fish.

Additionally, the review examines the current regulatory frameworks (such as the Pesticide Registration and Control Proclamation, Labor Proclamation, and Environmental Pollution Control Proclamation) and management strategies to mitigate pesticide pollution in Lake Tana, its tributary rivers, and associated wetlands. Afterward, critical practice gaps are identified, and recommendations for future research directions and policy interventions are proposed.

*Keywords: Agricultural intensification, Ecological Status, Lake Tana Sub-basin, Pesticide Pollution*

#### **4.11.P-Tu441 Challenges in Developing Brazilian Scenarios for the Environmental Risk Assessment for Birds and Mammals Due to the use of Pesticides**

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The adoption of an environmental risk assessment methodology that has been developed by another country requires, preferably, the adaptation of exposure scenarios to meet the local reality. Climatic conditions, soil characteristics and the presence of wild animals in agricultural crops, for example, must be observed.

The Brazilian government, represented by the Brazilian Institute of the Environment and Renewable Natural Resources, has historically used the United States Environmental Protection Agency's Terrestrial Residue Exposure model in its environmental risk assessments for birds and mammals due to the use of pesticides. However, the use of scenarios that were obviously built for conditions different from those in Brazil brings uncertainty regarding the degree of protection afforded to Brazilian animals.

Thus, in recent years the Brazilian government has tried to adapt already established risk assessment methodologies for birds and mammals based on national databases, with a main focus on that developed by the European Food Safety Agency. To this end, data such as residues in food items and sightings of birds and mammals in agricultural landscapes were collected. There are several challenges to be overcome, but it is possible to say that the greatest difficulty is related to the survey of residues, as specific studies are needed so that they can be used in exposure scenarios.

#### **4.12 Statistics for Ecotoxicology and Environmental Fate – From Tried and Tested over New and Exciting Methods to Machine Learning**

##### **4.12.T-01 The Lack of Comparability in Machine Learning Studies for Environmental Hazard Assessment**

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The application of machine learning (ML) models holds promises for many domains of research. However, like classical experiment-based research, ML-based science comes with its specific set of pitfalls. Several branches of ML-based research in parallel are working on fostering awareness and implementing best practices that improve repeatability, comparability, and the quality of research in general. However, ecotoxicology seems to lag behind with the adoption of these measures like commonly used benchmark datasets and reporting standards. We assess the comparability across studies in our field by defining a set of comparability criteria that need to be met in order for the findings of studies to be comparable to each other: 1. Same dataset, 2. Same dataset processing and cleaning, 3. Same dataset splitting, 4. Same performance metrics, 5. Reporting of code & formulae of performance metrics calculation. We review literature from the latest 10 years, that used QSARS and machine learning methods to predict ecotoxicological outcomes and evaluate their comparability in accordance with these criteria. Our findings suggest that extremely few studies meet all of these criteria and can be considered comparable. We highlight the importance of implementing best practices as a necessary prerequisite for the progression of ML as a useful tool for ecotoxicological research.

##### **4.12.T-02 Under What Circumstances Is CPCAT Suitable for Field Data?**

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The Closure Principle Computational Approach Test (CPCAT) is a novel statistical approach that was initially designed for conducting multiple testing of reproduction data in the field of ecotoxicology. It has been used for analyzing count data, specifically for NOEC/LOEC analysis, in various ecotoxicological studies, with a particular emphasis on soil field studies. However, it should be noted that from a statistical standpoint, CPCAT is considered a relatively new method. There are some radical differences from the statistical approaches normally used in ecotoxicology.

In this talk, we report an in-depth analysis we did of the methodological properties of CPCAT and the implications for false positives and false negatives. The crux of the matter is the underlying assumption about Poisson distribution. The inherent characteristics of the Poisson distribution assume that the mean is equal to the variance, indicating that these two parameters are not independent. As a consequence of this, the significance of observed differences in CPCAT relies on the sample mean rather than the observed signal-to-noise ratio. Furthermore, the statistical power of CPCAT is heavily influenced by the absolute abundance of organisms and the underlying distribution of the ecotoxicological data. We found that when data is under-dispersed, CPCAT has many false negatives; similarly, with high absolute abundance, CPCAT may have under some certain circumstances more false negatives than other methods. In contrast, when data is over-dispersed, CPCAT has a very high rate of false positives, under some circumstances over 50% false positives. Moreover, CPCAT can only handle simple study designs, thus, repeated measures, nested designs, split-plot designs cannot be analyzed properly using CPCAT.

We conclude when data follows a Poisson distribution, CPCAT may be used as long as the study is a simple one-factor design. In all other circumstance, extreme caution should be taken before relying on CPCAT results for regulatory purposes.

#### **4.12.T-03 BMD estimation using model averaging for Avian Reproduction Studies**

*Xiaoyi Sopko, Corteva Agriscience*

The purpose is to summarize the simulated learnings of benchmark dose modeling for avian studies via various models fits, and various model averaging techniques of frequentist models; and assess the use of prior information in Bayesian models. From the simulated learnings, one can appropriately select on the conditions in which a BMD can be successfully estimated. The simulations cover various dose response shapes, variances, spacing of application rates, and number of application rates. Under conditions where one of more observed dose groups is close to the true BMD estimate, Bayesian models with default priors can provide much better estimates than the frequentist models as well as tighter confidence intervals in cases where limited dose groups are available under specific set of conditions (e.g. largest treatment is greater than 10% effect, etc., decent model weights). Strongly informative prior does not always perform better than Bayesian models with default priors or weakly informative prior. A set of established, published priors would prevent from creative modeling. BMD is an effective tool with benefits surpassing the use of single models to calculate ECx. However, there are certain conditions and requirements that must be satisfied in order to use model averaging successfully. We explored and summarized appropriate conditions and requirements for estimation of BMD using both the frequentist methods and the Bayesian methods.

#### **4.12.T-04 Mother-to-Egg Transfer of Chemicals in Reptiles Using Orthogonal Regression to Account for Uncertainty in Observations**

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To support exposure assessment of early life stages, we aimed to develop a predictive model to quantitatively link concentrations of chemicals that are measured in tissues of mother and their offspring. This model captures the relation between two continuous, quantitative variables that are both observed with a comparable degree of uncertainty. Such data violate a basic assumption of classical linear regression, namely the existence of an independent variable that is measured without error and used to explain another dependent variable. To overcome this limitation, we set up an orthogonal regression model which explicitly accounts for uncertainty in both variables. The model accounts for uncertainty in measured variables by applying a Gaussian error distribution on both variables and constraining their range in relation to the population mean. Alternative formulations were developed to improve model performance and parsimony. Parameters were then calibrated using Bayesian inference to ensure that the full parameter uncertainty could be propagated into the probabilistic model predictions. Model alternatives were compared for their goodness-of-fit and predictive performance during 3-fold cross-validation. To support model development, we utilised a large database that resulted from a systematic search, extraction, and integration of scattered data from the scientific literature regarding measurements of organic pollutants in paired samples from mother and offspring tissues across several reptile species. We specifically focussed on reptile species as a group which has traditionally been underrepresented in ecotoxicology and chemical risk assessment despite its potential high sensitivity to maternally transferred pollution burdens resulting from their unique life history traits. After identifying the most suitable model alternative (i.e., base model structure), further model extensions were made to investigate ecotoxicological questions: 1) is maternal transfer influenced by the magnitude of the mother's body burden; 2) are there taxonomic patterns in maternal transfer; and 3) does the persistence of a compound influence its maternal transfer. The database, model, and new scientific insights provide the tools and knowledge to enable quantitative integration of maternal transfer-mediated early life exposure in chemical risk assessment.

#### **4.12.P Statistics for Ecotoxicology and Environmental Fate – From Tried and Tested over New and Exciting Methods to Machine Learning**

##### **4.12.P-Tu442 Improved and Harmonized Statistical Approaches in Non-Mammalian Endocrine Disruption Testing: Recommendations for Improved Data Analysis in OECD TG 231 and 229/230**

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This presentation aims to address the challenge of inconsistent statistical approaches in non-mammalian endocrine disruption (ED) testing, specifically focusing on the Amphibian Metamorphosis Assay (AMA) and Fish Short-Term Reproduction Assays (FSTRA) as outlined in OECD TG 231 and 229/230. By harmonizing statistical analysis methods, we can improve the reliability and comparability of results obtained from these assays.

To achieve this, we compiled a comprehensive list of endpoints measured in both assays and identified the direction of possible effects. We also considered endpoints mentioned in the Office of Pollution Prevention and Toxics (OPPTS) guidelines and addressed other critical aspects, including the choice of control for effect comparison and calculation of the coefficient of variation (%CV) for mean measured concentrations. Our recommendations were derived from OECD's document on statistics in ecotoxicology and relevant scientific literature.

By establishing a consensus with industry stakeholders, including the CropLife Europe (CLE) Statistical and ED groups, we aim to advocate for the inclusion of these recommendations in the updated OECD guidelines. This collaborative effort will enhance the quality of statistical analysis not only in studies involving Plant Protection Products but also in the growing number of ED studies required for REACH registration.

This research is highly relevant as it addresses the need for standardized statistical approaches in non-mammalian ED testing. Implementing these recommendations will advance the field by improving the reliability and comparability of data across different laboratories. Furthermore, the application of harmonized statistical methods will contribute to more accurate assessments of the endocrine-disrupting potential of chemicals, ensuring the safety of both humans and the environment.

#### **4.12.P-Tu443 Activities to revise the OECD Document No. 54 (and ISO/TS 20281) on statistical analysis of ecotoxicity data**

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The OECD Document No. 54 (2006) "Current approaches in the statistical analysis of ecotoxicity data: a guidance to application" and its companion ISO/TS 20281 describe the main statistical methods used for data analysis from ecotoxicological studies. However, some of the approaches described in OECD Document No. 54 are no longer considered as state of the art. In recent years, new methods have been published and other statistical tests have been adapted to the specific data characteristics in ecotoxicology. A revision of the OECD Document No. 54 and ISO/TS 20281 is urgently needed, as the choice of statistical method and its application has a direct impact on all OECD and ISO test guidelines and thus on the assessment of effects of currently regulated chemicals on (non-target) organisms.

With this contribution, we present a project initiating this revision process. The project aims to revise the OECD Document according to the current state of science and technology. Moreover, further development in analyzing ecotoxicological biotests is focused by harmonizing advances in statistical approaches and methods.

The project background, the aims of the project and first results are presented. Central issues are the update of guidance on NOEC determination by including new approaches with specific consideration of current methods for non-parametric and parametric approaches (e.g. for non-normally distributed variables). In this context, more precise guidance on the assessment and interpretation of statistical power and the underlying distribution is sought. Regression approaches for EC<sub>x</sub> calculation will be expanded and the use of reliability criteria for the derivation of EC<sub>10</sub> values with its confidence intervals (and related concepts) as an alternative statistical measure to the NOEC concept will be outlined.

Further development will take place in collaboration with an accompanying group of statisticians. The engagement with international experts ensures a comprehensive understanding of emerging statistical approaches, guaranteeing that the revised guideline remain at the forefront of methodological advancements.

The outcomes are anticipated to contribute to the refinement of global standards, fostering improved decision-making processes in the assessment and management of chemical risks to ecosystems.

#### **4.12.P-Tu444 MOSAIC\_ssd: a turnkey web tool to facilitate Species Sensitivity Distribution analyses**

*Miléna Kaag and Sandrine Charles, PhD, HDR, Umr Cnrs 5558, University Claude Bernard Lyon 1, France*

The use of Species Sensitivity Distribution analyses (SSDs) is acknowledged since the 1980s as a key and relevant decision support tool in environmental protection and management. To this day, statistical methods and regulatory applications continue to evolve. Ten years ago, the MOSAIC\_ssd web tool was made available with the specificity of allowing the use of interval-censored data. Since then, this tool has gained in popularity and started being used around the world, in all sectors: academic, regulatory, and industrial, where SSDs prove useful, relevant and/or mandatory. Consecutively to the successive updates and added features of the MOSAIC web platform, the MOSAIC\_ssd tool also needed a revampment, some improvements, and additional features. This poster presents the new version of the MOSAIC\_ssd service now available either as an R-package (ssd4mosaic) which allows an autonomous use from a local installation, or as an online turnkey web tool (<https://mosaic.univ-lyon1.fr/ssd>) that provides formatted results in just a few clicks. The poster specifically shows the new graphical interface separating the inputs from the outputs in different tabs; the various functionalities improvements such as the possibility to group and label species observations; and the new functionalities such as the choice of the uncertainty quantiles or the propagation of the uncertainty along the fitted probability distribution curve.

#### **4.12.P-Tu445 Can custom-written codes be GLP compliant?**

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Statistical programming tools such as SAS, Matlab, Python and R have revolutionized data analysis processes, particularly for large datasets. Additionally, KNIME (Konstanz Information Miner), a popular low-coding, free, analytic platform with R and Python integration, offers great future potential. The free open-source system R has become a powerful tool by providing greater flexibility and innovation in communicating data by using various packages with different functionalities. R packages

are constantly updated, which is great for statistical advancements and improvements, but is problematic for use in and validation for Good Laboratory Practice (GLP) studies. Validation for GLP compliance requires proof that the program is operating correctly, a risk assessment to determine the probability that errors or misuse could occur, and documentation and evidence of change and version control. The outcome is a high level of assurance that the program and functions are fit for purpose and results are reliable and trustworthy. Whilst there are documents setting out coding standards and best practices to ensure that the data processing and analysis can be clearly followed by another user, this does not fulfil the requirements of GLP validation. There is a lack of clarity and guidance on how such customised tools e.g., codes/workflows, can be validated for GLP compliance. Although OECD guidance on computerised systems has been revised to address more sophisticated data analysis, customised systems are classified as the highest risk level. Thus, the validation process poses a significant challenge. Nonetheless, there are major advantages of coding and building data processing pipelines regarding the transparency of data handling and analysis, particularly when using open-source software such as R and KNIME. An R script or a KNIME workflow can be annotated to explain each data handling step and it can be re-run by any user on any computer, resulting in high reproducibility of the data journey. Using KNIME, together with coding software, increases the readability and understanding of each step (node) within a workflow and brings together separate data silos (loading, processing, and analyses). In this modular approach, each node or workflow can be individually validated and archived.

This poster will provide an overview on the issues of validation of programming tools, offer some potential solutions and ask some questions to open up discussions in the SETAC community.

#### **4.12.P-Tu446 ECX estimation for Hormesis-like Effects**

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The risk assessment of chemicals uses ecotoxicological endpoints based on concentrations that cause a certain effect on test organisms. In acute tests systems, the EC<sub>50</sub> (i. e. concentration causing 50 % effect) is commonly used, while chronic tests are expressed in so-called No-Effect Concentrations (NOECs) or EC<sub>10</sub> values. The uncertainty around EC<sub>x</sub>-values is commonly quantified by confidence intervals. In some cases, hormesis occurs as biological (Hormesis effects) or ecological response (Hormesis-like effects). A hormesis has a biphasic dose–response relationship: Beneficial effects are present at low concentrations and decreasing effects dominate with increasing concentrations. Several examples of these phenomenon have been observed on different test parameters for plants and soil organisms in both, lab and field studies.

Non-linear regression models are widely used to fit a reasonable biphasic curve to the test data, with generally a good model performance (Brain & Cousins 1989, and Cedergreen et al. 2005). In these hormesis models, the EC<sub>x</sub> is estimated based on the part of the fitted curve behind the turning point. Therefore, only the decreasing response is evaluated and not the positive effects (i.e. hormesis-like). This approach violates the regulatory requisite that a substance should not have any effect on the organism.

Our aim is to include the hormesis-like effect in the determination of endpoints for use in the risk assessment. Therefore, we quantify the difference between the classically used methods and our newly developed approaches, both in value and uncertainty. In this comparison, the turning point of the curve between the control response level and the point of the maximum (positive) response will be determined as the starting point for the estimation of the EC<sub>x</sub>.

Another approach to identify the starting point is to use a two-sided hypotheses test to identify the NOEC and LOEC values. Hereby the test with the highest statistical power for that specific type of distribution will be selected. The linear interpolated midpoint of the logarithmic concentration values of the NOEC to the LOEC will approximate the starting point of the curve area for the EC<sub>x</sub> estimation. In case there is no NOEC, i.e. the lowest concentration already signifies an effect, the midpoint of the control and the LOEC will be used. Exemplarily results show that the EC<sub>x</sub> values of the approaches could differ up to 4 times, which would impair the risk evaluation.

#### **4.12.P-Tu447 Equivalence Testing in Honeybee Semi-field Testing and its Consequences**

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In 2023, EFSA released the *Revised guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)*, which will likely be implemented in 2024. Besides many fundamental changes to pollinator environmental risk assessment (ERA), the statistical evaluation of honeybee (*A. mellifera*) semi-field and field tests has been re-envisioned based on the equivalence testing framework. In contrast to standard null hypothesis significance tests which are built for demonstrating the presence of effects, equivalence tests aim at demonstrating that effects are smaller than a pre-specified threshold. While this approach is successfully used in pharmacology, it is unclear how it will transfer to honeybee (semi-)field data in the ERA context, given the higher intrinsic variability of these test systems and the 10% effect size set as specific protection goal.

In the present study, we use negative control data of Bayer AG semi-field honeybee tests to characterize the test system's inherent variability for the main endpoint colony strength (i.e. number of bees). Based on the estimated variability, we use data



simulations to investigate which true effect sizes can be reliably (i.e. with high enough power) detected as < 10% within the equivalence testing framework. We discuss the consequences of these findings for the experimental setup of semi-field honeybee studies and for the envisioned pollinator ERA.

#### **4.12.P-Tu448 First Experiences With the Statistical Methods From EFSA's Revised Bee Guidance Document**

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In May 2023, EFSA (European Food Safety Authority) released a revised guidance document (GD) on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). The document outlined methods for exposure and effect assessments using a tiered approach. In the absence of more detailed worked examples and an official calculator tool, we have been testing these methods for effect assessment to several lower tier studies.

Based on our evaluations we identified several challenges to comply with the revised EFSA GD when using common statistical software tools for evaluation of GLP studies. For example, some software tools do not apply the same model parameterization as described in the supplementary information to the GD. The inflection point, especially for the asymmetric Weibull models (which is different from the LD(D)<sub>50</sub>), is not given explicitly in the output. Consequently, a logarithmic conversion would be needed for use in EFSA's PIE calculation equations. Another discrepancy is that some software tools use a different logarithmic base (e.g. log<sub>10</sub>) from the natural logarithm employed by EFSA. This results in incompatible slope values for dose-response models for the calculation of the predicted individual effect (PIE) level. Furthermore, the mostly used GLP software does not provide all four dose-response models (i.e. Log-logistic, Log-normal, Weibull-1 and Weibull-2) as required by EFSA. Additionally, some software tools only offer 2-parameter models in combination with Abbott's correction for data from studies with non-negligible control mortality, whereas the approach proposed in the revised EFSA GD requires the use of 3-parameter models, which are statistically more appropriate.

Importantly, the EFSA recommendations about the calculation of PIE is not explicit about how to include control mortality in PIE. In this poster, we propose a method to control for control mortality using both Abbott's formula and the 3-parameter models. We base this method on the EFSA BMD guidance which uses a similar approach for calculating BMR. The suitability of the method will be discussed.

#### **4.12.P-Tu449 Predicting Fish Sensitivity Across Species and Chemicals: Combining Physiological Variables with Chemical Hydrophobicity**

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A major challenge in ecological risk assessment is estimating the toxicity of chemicals on a diverse set of species without performing species-specific toxicity tests. A potential way to tackle this problem is by extracting as much mechanistic understanding possible from currently available toxicity data. Here, we explore the potential and suitability of linear mixed effect (LME) models to explore the sensitivity of fish across both species and chemicals simultaneously. For this, we combine both chemical hydrophobicity and physiological properties (represented by dynamic energy budget parameters) in LME models with a varying level of complexity. We find that differences in sensitivity are larger between chemicals than between species, and that hydrophobicity is therefore the strongest predictor when looking across both chemicals and biological space simultaneously. After zooming in on individual chemicals, we found that physiology explained up to 99% of the variation in species sensitivity. Our approach illustrates how both chemical and biological properties may drive species sensitivity and how these two dimensions can be combined into one statistical model, allowing for more realistic predictions of effects across species and chemicals without the need for additional animal testing.

#### **4.12.P-Tu450 XEREDAR, an R-package for automated statistical analysis of embryo assays for studying endocrine activity**

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The statistical characteristics of ecotoxicological New Approach Methodology (NAM) assays for studying endocrine activity using aquatic vertebrate eleutheroembryos (XETA, RADAR, and REACTIV) differ from those of standard Tier-1 ecotoxicological studies. In these assays, all concentration groups are nested in the replicates (referred to as runs), while in standard Tier-1 studies, the replicates are usually encapsulated in the concentration groups. Ignoring this difference in dependency structure during the statistical analysis violates the basic statistical notion of independence and can potentially lead to erroneous conclusions.

Unfortunately, the statistical section of OECD Test Guideline No. 251 (RADAR) recommends disregarding the dependency structure, and the recommendations in OECD Test Guideline No. 248 (XETA) are at least ambiguous in this regard. However, Annex 13 of the same test guideline provides detailed statistical recommendations using a mixed ANOVA approach.

The developed xeredar R-package is an open-source tool for automated statistical analysis of ecotoxicological NAM assays, specifically for assessing endocrine activity, while considering the correct dependency structure through a mixed ANOVA. The validation of this R-package involved comparing the outcomes of the 36 XETA validation studies, with the results from the xeredar analysis. It was found that the results of using xeredar are congruent to the results presented in the original validation document. Additionally, a power analysis for the REACTIV assay (currently under development at OECD) highlights the consequences of ignoring the dependency structure, including inflated type I (false positive) and type II (false negative) errors. Ignoring the dependency structure reduces power and increases type I error compared with the mixed ANOVA approach.

Overall, xeredar is a user-friendly R-package that aims to standardize the statistical analysis of the XETA, RADAR, and REACTIV and provide reproducible results, thereby promoting the use of NAMs for ED assessment in regulatory ecotoxicology.

#### **4.12.P-Tu451 After dark, all CATs are leopards - Casting Light on the Use of CPCAT (Closure Principle Computational Approach Test) in Non-Target Soil Organism Assessments**

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Recently, the ‘Closure Principle Computational Approach Test’ (CPCAT) was developed as a method that should overcome the shortcomings of NOEC/LOEC application in ecotoxicological pesticide risk assessments. CPCAT handles abundance data characterized by non-standard statistical properties, such as low abundances, non-normal distributions, and heteroscedasticity without restrictions. The method is recommended as a valuable tool for non-target soil organism assessments under field conditions. In this context, a common approach for guidance conform earthworm and mesofauna data collection is to transform low-resolution sampling data to a square meter scale before performing the statistical analysis.

Our research aims to evaluate the suitability of CPCAT for analysing abundance data of non-target soil organisms under field conditions. Particularly, we address the sensitivity of CPCAT to this data transformation and its implications for the reliability of the results. Specifically, to assess the performance of CPCAT, we used simulated data that covers a range of different data transformations. Therefore, we first generated typical field data sets for Collembola abundance. Then, we examined the behaviour of CPCAT when applied to such abundance data, considering data transformation and the subsequent change of data properties such as data dispersion and effect size.

Our study reveals a concerning sensitivity of CPCAT to data transformation, leading to an unexpected inflation in the false-positive rate which is correlated with the extent of the transformation applied. Transformation magnified the data's effect size as well as the dispersion within the data in violation of CPCAT's assumption regarding Poisson-distributed data. This raises concerns about the robustness and reliability of CPCAT under the current recommendations and underscores the need for caution when applying CPCAT to abundance data.

Overall, our study highlights the limitations of CPCAT in this specific context, emphasizing the importance of considering the applicability and reliability of statistical methods for ecological data in general. These findings have practical implications for researchers and environmental assessors in ecotoxicology and beyond, emphasizing the need for careful choice of methods for data analysis and potential improvements in statistical approaches and guidelines to enhance accuracy and reliability.

#### **4.12.P-Tu452 Promoting Statistical Rigor, Reproducibility, Traceability, and Flexibility in Regulatory Environments: A Comprehensive Showcase**

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Pesticide regulations aim to prevent unintended adverse effects to human, animal health and the environment from the use of plant protection products. Proper statistical analysis of study data is crucial in deriving the hazard endpoints, forming the foundation for risk based regulatory decisions. Like the generation of quality test data, quality statistical analysis is imperative for instilling confidence in regulators regarding the validity and appropriateness of the analyses they review and the following regulatory decision-making. The minimum level of acceptance standards should include reproducibility and traceability. How reproducibility and traceability for statistical analysis can be ensured, and how the implementation and use of appropriate analysis procedures can be guaranteed are essential questions. Additionally, adopting and implementing a new statistical methodology in a controlled and verified way poses challenges. Moreover, how should the various analysis tools (including “cloud” computing) and the retention of electronic data be treated under the context of GLP (Good Laboratory Practice) principles for computerized systems? In this presentation, we will address these key questions by showcasing an ecosystem with a platform providing both a file management system and an application hosting service featuring multiple shiny apps for various statistical analysis workflows.

#### **4.12.P-Tu453 Graphical evaluation of the results of a nonlinear dose response parameter estimation in R and SAS**

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EC<sub>x</sub> (Effective Concentration x) calculations are widely employed in environmental risk assessment to quantify the dose-dependent relationship between a stimulus and its biological response. In regulatory contexts, EC<sub>x</sub> is defined as the concentration or dose of a substance that induces a specific effect, typically expressed as a percentage change relative to the mean control response in regulatory context. To calculate the EC<sub>x</sub> values, the fitting of a dose-response curve to experimental data is necessary.

Quality assessments pivotal for evaluating the reliability of the results. A graphical evaluation can be instrumental in identifying factors contributing to poor fitting, particularly when employing nonlinear regression fits.

Various dose-response models assign specific meanings to parameters, with slope (parameter b), lower plateau (parameter c), upper plateau (parameter d), and reflection point (parameter e) representing distinct aspects of the curve.

When measured values are absent in certain curve regions, optimal parameter adjustments become challenging. The absence leads to poor p-values for the individual parameters (p-value >  $\alpha$ ), also affecting the lack of fit test results. Exploring graphical representations of the dose-response curve and measured values provides insights into the reasons behind suboptimal p-values and the actual usability of the fitted dose-response curve.

Different estimation methods or programs will lead to different results, if parts of the curve are not represented by measured values. In this case, there are different parameter estimations that can lead to similar fitted curves. In some cases, the fitting is no longer possible for a particular optimization algorithm without specific adjustment. However, if the measured values represent the curve well for all areas, the choice of the estimation methods or program has little influence on the result of the parameter estimation.

These relationships are illustrated with graphics showing test data and fitted dose-response curves. The results of different estimation methods and programs for the data sets are included to make clear how the graphics can be used for an interpretation of nonlinear regression results. The examples come from different growth inhibition test data with *Myriophyllum spicatum* from laboratory studies. The non-linear models Log Normal, Weibull type 1 and 2 with three or four parameters are used to fit dose-response curves.

#### **4.12.P-Tu454 Digital Environmental Data Management System; Easier Said than Done**

*Alexandra Duguay, Rio Tinto, Canada*

At Rio Tinto, one of our core business objective is to achieve impeccable Environmental, Social and Governance (ESG) credentials. To achieve this, a strong, reliable corporate Environmental Data Management System (EDMS) is required. Given the breadth and scale of our operations, which span across multiple jurisdictional reporting requirements and include multiple data types and values (both numerical and non-numerical), the collection, storage, organization, analysis of this data represents a significant endeavor.

With this work, we present how we have successfully implemented one of the pre-eminent ESG software packages at the Rio Tinto Aluminum Atlantic Quebec operations hub. The EDMS currently integrates environmental data related to Green Houses Gases, Air and Water quality, weather patterns and several other key metrics from all the Saguenay-Lac Saint-Jean business units including the alumina refinery, five smelters, six hydropower plants, rail network, international port as well as from the Arvida Research and Development Centre. We also present the lessons learned from the digital conversion of such a complex and diverse set of environmental data as an opportunity to highlight aspects that could have resulted in challenges.

As environmental specialists, we are all very familiar with the utmost importance of unbiased scientific data and information that serves as a foundational element for decision making. We believe that sharing the key outcomes of this work can help organizations gain a greater understanding on maintaining and improving environmental data quality through tools that provide better management, and mitigation of various environmental challenges. Furthermore, this work can support broader use of EDMS, particularly in multifaceted large and complex sites such as ours.

#### **4.12.P-Tu455 A comprehensive screening approach to assess similarity to Substances of Very High Concern**

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Over 350,000 chemicals and mixtures are currently being used and emitted into the environment. For many of these chemicals we lack sufficient toxicity information to assess and evaluate their toxicological profile. As *in vivo* – and to a lesser extend *in vitro* – toxicity tests are cost and time consuming, the use of *in silico* methods can be beneficial to fill the existing datagaps. In such an approach, it is crucial to identify the chemicals of highest concern. This particularly includes chemicals with C, M, R, PBT/vPvB and ED properties, which are also known as Substances of Very High Concern (SVHC) in the EU. To facilitate the identification of SVHCs, we recently developed models that evaluate the structural similarity of substances with unknown properties to known SVHC [Wassenaar et al. 2022; <https://doi.org/10.1002/jcc.26859>]. Substances that are structurally

sufficiently similar to known SVHCs can be prioritized for further evaluation, as a high resemblance in chemical structure can be an indication of comparable effects ('similar property principle').

Although this structural similarity model works great at identifying chemicals that exhibit (small) structural differences with known SVHCs, it does not address (dis)similarities in toxicokinetic or biological activity. Extension of the SVHC similarity tool with other aspects of toxicokinetic and biological similarity, such as physicochemical properties and mode of action, can support and increase the users' confidence in the similarity predictions.

As such, in this study we extended the chemical 'global' similarity to site-specific similarity (i.e. structural alerts), and considered toxicokinetic and biological similarity in the form of a physicochemical profile and a bioassay-based profile. Although the individual similarity profiles do not outperform the global similarity model, combining these models in an extended similarity approach result in much more robust predictions, giving information for (additional) mechanistic reasoning that support (or disprove) the initial prediction based on (only) structural similarity. It particularly improves interpretability and translatability to the broader chemical universe and towards relevant follow-up evaluations. Consequently, these model additions could enhance the accuracy of identification of potential SVHCs. In future, the developed models could be optimized to other chemicals and effects of concern to contribute to the development of safer chemicals.

#### **4.12.P-Tu456 PEPPER: Machine Learning for Predicting Environmental Persistence of Pollutants under a Unified Framework**

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Understanding the fate of environmentally problematic substances, particularly their microbial degradation, is crucial for effective chemical risk assessment and sustainable chemical development. This study introduces PEPPER, an open-source library providing a standardized approach for handling and sharing degradation data. PEPPER aims to establish a benchmark for modeling advancements, incorporating transparent data curation and well-established machine learning methods. It addresses small datasets with uncertain experimental values, focusing on two biodegradation endpoints: primary biotransformation half-lives (DT50) and breakthrough during wastewater treatment.

Currently our curated database includes 536 chemicals and 18 plants, the largest of its kind. We present results of several modelling attempts using this dataset and several combinations of regressors and chemical descriptors. The study highlights the impact of data curation strategies and dataset size on model performance, emphasizing the need for additional chemically diverse training data for improved predictions.

We plan to expand the application of PEPPER to biodegradation in soil and sediment, with a focus on incorporating uncertainty to enhance model predictive and informative capabilities. This work represents a significant step towards a better understanding of the fate of micropollutants in the environment.

#### **4.12.P-Tu457 High performance computing and neural network in support of toxicokinetic-toxicodynamic modelling for the understanding of mixture effects**

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Plant protection products are formulated to maximise efficacy to control target pest species, including mixtures of active ingredients, while at the same time limiting risks to non-target species. Being able to predict joint effects of active ingredients or of active ingredients together with formulation agents, is of great importance to both the chemical industry and risk assessors.

As a consequence, having a tool to predict potential interactions without having to test all combination in the lab would increase the efficiency of selecting optimal combinations of active ingredients and formulation products for product development as well as decrease animal testing. As toxicity is a process in time, assessing effects over time gives valuable information on the toxicity of both single chemicals and mixtures. Time variable effects can be assessed with toxicokinetic (TK) and toxicodynamic (TD) models. The mixture toxicity concepts (Concentration Addition and Independent Action) have recently been implemented in survival TKTD models. Here, we provide a standalone pipeline coupling TKTD mechanistic models with Neural Network (NN) benefiting from both worlds: (1) the TKTD approach to fit experimental data (under a Bayesian framework) on active substances alone and in combination with other active substances or additives; (2) using the NN to map potential interactions between active substances in combinations or in formulations. Ultimately this helps identifying chemical mixtures which deviate from the reference models and predicting interactions of new combinations and formulations, not yet tested.

Our model is currently tested based on 99 acute toxicity (survival) studies with fish (OECD 203 standard), grouped into 4 datasets (comprising 38, 19, 21, 21 OECD 203 studies, respectively), with 1 to 3 active substances for each study. Each of the

4 datasets allowed studying the interactions between 5 to 6 different active substances present in varying concentrations in the formulations. Here, we present a first analysis demonstrating the robustness of the model, together with preliminary results on the most frequent type of interaction.

#### **4.12.P-Tu458 Machine Learning to help identifying chemicals**

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Introduction: As the volume and complexity of analytical data grow, conventional software often falls short in effectively analysing it. Consider, for example, the challenges in identifying polymers through infrared spectra that are compromised by impurities or degradation. This is where machine learning can make a significant difference. Here, we will showcase two test cases that underscore the advantages of machine learning. Our findings suggest that not only does machine learning enhance the accuracy of results, but it also expedites the analysis process. As a result, this approach offers the potential for significant cost savings. We will demonstrate this on two examples, polymers analysis using LDIR (laser direct infrared) spectroscopy and identification of salt solutions with EIS (electrochemical impedance spectroscopy).

Polymer analysis: A notable correlation between the number of training spectra and model performance is seen. As sample numbers grow, most model performances improve, with minor exceptions between 30-50 samples. Thus, scarce training samples can compromise optimal results. Distinguishing by performance, Model 1 excels with larger datasets (>30 samples), peaking at 40, while Model 2 matches this only at 100 samples. Model 3 slightly surpasses Model 4 with smaller datasets, but both need more samples for enhancement. Given computation time and peak performances by Models 1 and 2, training with over 100 samples per polymer class was deemed unnecessary in this research.

Salt solution analysis: We plotted the impedance data derived from the Electrochemical Impedance Spectroscopy (EIS) in a polar coordinate system. The visual representations of this coordinate system were employed to train a CNN model. Subsequent identifications used this trained model. The resultant confusion matrix indicates optimal performance with a limited dataset. Future work will focus on expanding both the sample count and the variety of salts under consideration.

Conclusions: (1) Even a basic model can prove its efficacy. A straightforward sub-KNN approach outperformed three other CNN models and taking less time to train. (2) Quantity of good samples is pivotal for training effective models. With a limited dataset, our data augmentation strategy led to a significant boost in performance. (3) While 2D-CNN models are not the best in this specific study, they appear promising for handling multidimensional spectroscopic data. Their potential will be explored further.

#### **4.12.P-Tu459 Linking Machine Learning to GC/MS Data: A New Approach for Simplifying and Facilitating Environmental Data Analysis**

*Day Powell, Tamas King, Ruoji Luo and Edison Cerda, Agilent Technologies, Inc.*

GC/MS data analysis is often manual, time consuming, and human reliant. There are many automated integrators available, however, most of the time a chemist will need to manually correct baselines, combine or split peaks, and eliminate false positive or negative peaks. The manual intervention is often resulted by contamination, matrix effect, insufficient separation, bad peak shape (such as fronting and tailing), or the nature of analytes.

A machine learning (ML) architecture, designed for GC/MS data processing software allows continuous “learning” of lab-specific methods for various testing services. The plug-in tool operates first in passive mode, building a ML model based on a chemist’s current data analysis workflow. Data analysis results are continuously monitored and fed into a training pipeline to generate a model using a deep learning neural network.

A ML model for the phthalates analysis in consumer products was developed. Another ML model targeting the analytes in the EPA 8270 method is under development and evaluation. Over 1000 samples were used to train the first ML model for phthalate analysis. Data were acquired in SIM/SCAN mode. During the phthalate analysis, isomeric compounds, such as diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP), result in broader peaks and irregular peak shape and usually require additional manual integration. The fully trained ML model can correctly integrate all phthalates investigated in this study, including DINP and DIDP. Retention time shift and change of qualifier ratio sometimes might occur during the GC/MS data acquisition, especially after the maintenance or column replacement. Both parameters were considered during the model training process. With the fully trained ML model both the quantifier ion as well as the corresponding qualifier ion(s) of each phthalate can be correctly integrated across the calibration range. The data analysis time was reduced to 1/5 to 1/4, when the manual integration step is replaced by the prediction of the fully trained ML model.

The GC/MS data processing plug-in tool with a continuously learning model provides reproducible and reliable results, drastically reduces number of manual integrations, therefore reduces the overall data analysis time.

#### **4.12.P-Tu460 Bio-QSARs unlock a new level of predictive power for machine learning-based ecotoxicity predictions by exploiting chemical and biological information**

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Practical, legal, and ethical reasons necessitate the development of methods to replace animal experiments, so-called new approach methodologies (NAMs). Computational methods such as machine learning (ML)-based quantitative structure-activity relationship (QSAR) models are widely considered a crucial pillar among NAMs. Recently, we introduced the Bio-QSAR concept for multispecies aquatic toxicity regression tasks. For training these models, not only chemicals are represented by a set of numerical descriptor features but also the included species. While first-generation Bio-QSARs for fish and aquatic invertebrates were already of high predictive power, we identified avenues for further improvement. First, we strengthened the data foundation of Bio-QSARs by expanding training datasets by a factor of ~20. We moved from pesticides to the whole chemical landscape as well as from freshwater to aquatic species in general and allowed for flexible test durations. To realize the latter, we used an ML algorithm that is capable of handling fixed and random effects, namely Gaussian Process Boosting. Second, we tested training Bio-QSARs using different sources of biological information besides Dynamic Energy Budget parameters by including taxonomic distances and genus-specific invertebrate trait information. Moreover, we assessed the addition of chemicals' mode of action, as a link between chemical and biological information. Third, we made the improved Bio-QSARs fully explainable by algorithmically correcting datasets for multicollinearity to allow for unbiased estimates of feature importance, which were based on a state-of-the-art approach from the field of explainable ML. Finally, we developed new methods to construct applicability domains that consider feature importance and tested their suitability to restrict predictions to samples the models are fit for. The developed second-generation Bio-QSARs are capable of flexible cross-chemical as well as cross-species predictions beyond levels present in training data and, to the best of the authors' knowledge, feature predictive powers unprecedented in multi-species models and tools for acute aquatic toxicity prediction (R-squared values of ~0.89-0.92). Moreover, they meet all criteria to be considered for regulatory purposes. The proposed NAM approach hence holds promise to benefit a range of applications in environmental risk assessment as well as research and development.

### 4.13 The Fate and Effects of Micro- And Nano-Plastics in Relation to Human Health Exposure

#### 4.13.T-01 21GRD07 PlasticTrace Metrological traceability of measurement data from nano- to small microplastics for a greener environment and food safety

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Plastic pollution is recognised as a severe anthropogenic issue globally, where complex physico-chemical transformation processes (such as aging, degradation and fragmentation) producing MPs and, subsequently, NPs. Several studies have reported the occurrence, analytical methods and toxicity of larger MPs (>100 µm) in the environment and food matrices. However, MPs (< 100 µm SMPs) and NPs (< 1 µm) in natural systems have been overlooked, primarily due to significant methodological challenges associated with their micro- and nano-specific properties. The present project, PlasticTrace, aims to address the urgent need for the development and harmonisation of methods for the chemical identification, physical characterisation and quantification of released small micro/nanoplastics (SMPs/NPs) in drinking water, food and environmental matrices, as required by the EU's Circular Economy Action Plan (CEAP). In this context, hyphenated and complementary analytical approaches will be developed, optimised, compared and harmonised, leading to the establishment of metrological traceability of measurements through inter-laboratory comparison validation studies. Novel and environmentally relevant SMP/NP reference materials will be developed within the project (e.g. Polyethylene terephthalate (PET), Polyethylene (PE)). International cooperation with key stakeholders such as EURAMET's European Metrology Networks, standards developing organisations (e.g. ISO TC 229, ISO TC 61, and CEN TC 249) and end users (e.g. food and drink producers, environmental monitoring programmes and health experts) will be considered as the basis for a European Metrology network. The need for efficient and reliable measurement infrastructure is required in support of (i) the European Chemicals Agency (ECHA)'s proposed restriction targeting intentionally added MPs in consumer products, (ii) the Marine Strategy Framework Directive (MSFD) which requires specific thresholds for litter types after harmonisation of the methodology, (iii) the new Drinking Water Directive (DWD) that mentions MPs explicitly, and (iv) the new CEAP adopted in March 2020. However, to support the CEAP and reduce plastic contamination, methods for SMP/NP identification in food and environmental matrices are needed. These methods need to be metrologically validated using appropriate reference materials to establish harmonised and traceable measurements of SMPs and NPs.

#### 4.13.T-02 Carcinogenicity of micro- & nanoplastics long-term exposure

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Micro- & nano-plastics (MNPLs) are considered emergent pollutants widely spread over all environmental compartments. There is evidence that humans can internalize these MNPLs through inhalation and ingestion and that the small size of the plastic particles may allow for absorption, systemic biodistribution and bioaccumulation. Despite of the fact that their biological effects are being intensively evaluated, their potential health effects in humans remain poorly understood. One of the most underdeveloped areas of study is the determination of the effects induced by MNPLs under chronic scenarios of

exposure, being carcinogenicity the most relevant in terms of risk. In this context, the present talk will focus on presenting the current science on MNPLs carcinogenic potential, giving special attention to the approaches developed and results obtained in the frame of the large-scale EU Project PlasticHeal ([www.plasticheal.eu/en](http://www.plasticheal.eu/en)). Together with the available literature, the set of obtained data supports a potential carcinogenic risk associated to MNPLs long-term exposure. On this basis of evidence, the need of more studies becomes evident. Key research questions and remaining knowledge gaps will therefore be discussed in benefit of future research and the full assessment of MNPLs carcinogenic risk.

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*Keywords* - Microplastics, nanoplastics, carcinogenicity, health, risk, effects, knowledge gaps.

#### **4.13.T-03 From in vitro to in vivo; a tiered approach to study microplastic dynamics and immunotoxicity**

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Background: Micro- and nanoplastics (MNPs) are omnipresent in the environment and pose a possible risk to human health. Humans are continuously exposed to MNPs and it is known they enter the blood stream via ingestion and/or inhalation. However, MNP immunotoxicity and their dynamics *in vivo*, remain poorly understood.

Aims and methods: We developed a high-throughput survival assay to quantify if microplastics affect cell survival in freshly isolated neutrophils and monocytes from human blood. Microplastics were also intravenously injected into mice to study neutrophil uptake and activation *in vivo*, as well as particle dynamics and organ accumulation over 30 days via flow cytometry.

Results: In the *in vitro* assay, spherical polystyrene beads (PS) of 10µm in diameter and 'wild-type' polyvinylchloride (PVC) (<1µm and 1-5 µm fraction) negatively affected human neutrophil survival, whereas PS 50nm, PS 1µm, 'wild-type' polyamide6.6 (PA6.6) (1-5µm and 6-10µm fraction) did not. After intravenous PS 1µm administration, we observed microplastic clearance from the blood within 16h, but increasing accumulation in the liver and spleen at day 14 and 30. We noted an initial neutrophil influx in the liver at 4h post-injection, which returned to baseline levels after 16h. Neutrophils that phagocytosed PS 1µm, exhibited an activated phenotype by increased expression of CD11b and shedding of CD62L. At day 14 and day 30 post-injection, PS 1µm mainly resided in CD11b negative immune cell subsets in the liver and spleen.

Conclusions: In our tiered approach, we assessed immunotoxicity of spherical and 'wild-type' plastics *in vitro*, guiding the selection of microplastics for *in vivo* testing. Although PS 1µm did not significantly reduce human neutrophil survival *in vitro*, intravenous PS 1µm accumulated in the mouse liver and spleen, inducing initial neutrophil phagocytosis and activation. Future work will also include intravenous 'wild-type' toxic PVC as based on our *in vitro* findings.

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#### **4.13.T-04 Influence of Simulated Gastrointestinal Digestion on the Toxicity of HDPE and PET Microplastics with and without V. parahaemolyticus Biofilm**

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Polyethylene terephthalate (PET) and high-density polyethylene (HDPE) are widely used in Europe for food packaging, therefore, HDPE and PET microplastics (MPs) end up in a variety of food and drink products, leading to possible health effects. Once in the environment, these MPs can be rapidly colonised by microorganisms like *Vibrio parahaemolyticus*, a pathogenic species that causes acute gastroenteritis and severe septicemia. In this work, we evaluated the potential toxicity of polydispersed 5 µm HDPE (D50, commercially available) and 130 µm PET (D50, top-down cryomilled by centrifugal mill) MPs with and without *V. parahaemolyticus* biofilm (HDPE, PET, HDPE+V and PET+V) and with and without an *in vitro* simulated human digestion system (SHDS) pre-treatment (HDPE-SHDS, PET-SHDS, HDPE+V-SHDS and PET+V-SHDS) to the human gastrointestinal (GI) system. For that, undifferentiated Caco-2 cells and Caco-2/HT29-MTX/M-cells co-culture were exposed for 24h to 1-100µg/mL of these MPs and after that, effects on cell viability, inflammation and barrier permeability and integrity were examined. Scanning electron microscopy (SEM) was used to analyse *V. parahaemolyticus* colonisation on MPs. The colony forming unit (CFU) assay was performed to determine the number of viable microbial cells in the bacterial biofilm. SEM analysis showed that after 3 days of incubation, *V. parahaemolyticus* colonised the MPs surface, however, the pre-treatment with SHDS decreased the number of viable bacteria on the surface of MPs. Results from *in vitro* exposures showed a decrease in cell viability and an increase in inflammation in Caco-2 cells exposed to MPs+V, whereas the

pre-treatment with SHDS reduced this effect. In the co-culture, none of the treatments caused cytotoxicity and slight effects were observed in terms of barrier integrity and permeability. Inflammation was increased in exposures to high concentrations of MPs+V pre-treated with SHDS. Overall, results indicated that HDPE and PET MPs may act as vectors for *V. parahaemolyticus* and induce important cellular responses, leading to damage in the human gut. However, simulated GI fluids (SHDS) may reduce the number of bacteria associated to MPs surface, influencing the potential toxicity of the bacterial biofilm. This work was supported by the EU H2020 Project “Plastics Fate and Effects in the human body” (PlasticsFatE) under Grant Agreement no. 965367.

#### **4.13.T-05 Developing Analytical Methods to Measure Exposure to Micro- and Nanoplastics in Early-life Matrices**

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Plastic can break down into smaller particles, called microplastics (<1 mm) and nanoplastics (<1 µm) (MNPs). The fate of these particles and their effect on human and animal health is still mostly unknown. Studies show environmental pollutants may have an adverse health effect on the developing fetus, which makes the human placenta an important matrix in researching MNPs' health effects.

We have established a successful digestion protocol for human placental tissue which enables the characterization of MNPs in these samples. As particle number, morphology and chemical composition can affect particles' toxicity, we combine (spectro-)microscopic techniques to enable in-depth analysis on the particle level. We spiked placental tissue with defined commercial MNPs, namely fluorescent polystyrene (FPS) model spheres, to test their stability and calculate their recovery rates throughout the digestion protocol. To ensure the proper characterization of MNPs, a successful digestion protocol needs to sufficiently remove the biological matrix, assure the stability of MNPs, and lead to a high and reproducible MNP recovery rate.

1 g of placenta tissue with known concentrations of FPS spheres was digested with several different enzymes and buffers. Digestion was followed by filtration over a 700 nm pore size glass fiber filter. The amount of tissue left on the filter and the color of the filter were used as indicators for the success of the digestion. MNP stability was determined throughout all steps of the most suitable digestion protocol with Confocal Fluorescence Microscopy (CFM). Particles were seen as stable when no deformation or agglomeration was observed and detection did not suffer from potential dye leaching. Next, recovery rates were measured with CFM by imaging droplets of the liquids after digestion, and before filtration.

Proteinase K and trypsin, both in TRIS-HCl buffer, were found most suitable for tissue digestion. FPS spheres were found back after digestion and remained stable under the conditions used in this protocol. FPS particles were recovered for 110% ± 9.6 (N=30). The next steps will include different plastic species and non-fluorescence-based analysis techniques, such as Atomic Force Microscopy or Raman microspectroscopy. This development in digestion methods for complex matrices is an important step in creating a risk assessment of MNPs for the unborn child.

#### **4.13.P The Fate and Effects of Micro- And Nano-Plastics in Relation to Human Health Exposure**

##### **4.13.P-Mo400 Ingested Nanoplastics Induced Histological, Genotoxic and Gene Expression Changes in Mouse Gastrointestinal Tract**

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Despite extensive research on nanoplastics (NPs) health impact, the implications of exposure to polyethylene terephthalate (PET) remain underexplored. In particular, there is limited understanding of its potential effect on the mammalian gastrointestinal tract (GIT), even though PET is widely used for cookware coating, leading to ingestion of PET-NPs. In this study, we utilised a C57BL/6 female mouse model to assess the effects of PET-NPs after single gavage exposure and compared them with nano-sized polystyrene (PS; 50 nm and 200 nm), which have been more widely studied. Evaluations were conducted at 1, 7, and 28 days post-exposure, focusing first on histopathological changes in the small intestine and colon, as well as mesenteric lymph nodes. DNA damages using the comet assay were also evaluated in the small intestine and liver. Finally, alteration of expression of genes related to inflammation, immune and stress responses, cell cycle regulation, tissue remodeling, DNA damage, and collagen synthesis were analysed in the small intestine via multiplex RT-qPCR. Overall, histological analysis did not show significant damage to the GIT organs. However, PS exposure resulted in lymphocyte depletion and increased B cell accumulation in mesenteric lymph nodes, suggesting activation of the immune system; while PET had no effect. We also found size-dependent genotoxic effects; notably, PS200 induced significant DNA damage in the small intestine, which resolved over time, whereas PS50 and PS200 caused sustained liver DNA damage at 28 days. There was upregulation of inflammation and stress response-related genes, which correlated with PS50 robust pro-inflammatory responses at the histological level, while PS200 stimulated genes related to stress and antioxidant defense mechanisms.



Conversely, PET exposure suppressed genes vital for cell cycle progression, collagen formation, and DNA repair, hinting at long-term impacts on tissue integrity and genetic stability, with an increased xenobiotic response observed by day 28. These findings underscore the health risks associated with NPs ingestion, particularly in terms of genotoxicity and immunological reactions in the gastrointestinal tract. Crucially, this research will significantly influence the development of policies and regulations, especially in relation to NPs exposure via food contact products for food safety purposes.

#### **4.13.P-Mo401 Immune Effects of Pristine and Microbially Contaminated PET Nanoplastics**

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**Background:** Workers in plastic processing and manufacturing industries may be exposed to airborne micro- and nanoplastics (MNPs). Due to their small size and low density, MNPs can deposit in the lower respiratory tract, including the alveoli. MNPs might carry microbial contaminants, especially those existing in waste management and recycling facilities, thereby posing a health risk when inhaled by workers in these industries. Moreover, microbially contaminated MNPs may interact differently than pristine MNPs in the lung. In the present study, we investigated cell viability and inflammatory cytokines after exposure to pristine or microbially contaminated polyethylene terephthalate nanoplastics (PET-NPs) using co-cultures of human lung epithelial cells and macrophages.

**Materials and methods:** Bottom-up synthesized PET-NPs from commodity PET pellets (PET c000, D50 = 77 nm) or recycled juice bottles (PET b001, D50 = 69 nm) were assessed for microbial contamination using Toll-like receptor (TLR) reporter cells expressing either TLR2 or TLR4. Submerged co-cultures of human alveolar basal epithelial cells (A549) and monocyte-derived macrophages (dTHP-1) were exposed to low (10 µg/ml) or high (100 µg/ml) concentrations of the PET-NPs for 24 hours. Cell viability was assessed using alamarBlue reagent, and gene and protein expression of inflammatory cytokines (IL-1B, IL-6, IL-8, TNF-α) were measured by RT-qPCR and ELISA.

**Results:** PET b001 activated both TLR2 and TLR4, indicating the presence of microbial contaminants from Gram-positive and Gram-negative bacteria, while PET c000 did not cause TLR activation. No change in cell viability was found after exposure to any of the PET-NPs in A549/dTHP-1 co-cultures. The PET b001 particles increased the gene expression of IL-1B, IL-6, IL-8 and TNF-α, but only IL-6 and IL-8 were increased in the cell culture supernatant. The highest concentration of PET c000 particles (100 µg/ml) increased expression and secretion of IL-8.

**Conclusion:** Our findings show that exposure to pristine PET-NPs increased IL-8 expression, indicating a potential to cause inflammation. However, this response was much stronger when exposure occurs in the presence of microbial contaminants, which increased the expression of all the four cytokines measured. Workers at workplaces where such MNPs are present may therefore have a higher probability of experiencing occupational health issues after exposure to airborne MNPs.

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#### **4.13.P-Mo402 Micro- and nanoplastics migration from food packaging into food: a systematic evidence map**

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In recent years micro- and nanoplastics' (MNP) research has expanded to investigating potential sources of exposure. Emerging evidence shows that MNPs are generated during normal and intended use of plastic food packaging or other plastic food contact articles (FCA). However, to date FCAs as relevant human exposure source to MNPs has received little attention.

To provide a first overview of the available evidence, we performed a systematic evidence mapping on MNPs present in foodstuffs originating from FCAs. By identifying the extent of MNPs in food stemming from plastic FCAs we lay the foundation for refining human exposure assessment and reducing exposure.

We searched several public databases for relevant studies published until the end of 2022, screened them for eligibility, extracted a wide variety of data on the FCA, the MNPs, and the medium they were analyzed in. Finally, we assessed the reliability of the studies, and, based on the highly reliable ones, discuss the current body of evidence for plastic FCAs being the source of these MNPs in human food.

We identified 104 studies that met our eligibility criteria from which we generated 601 database entries. With more than 50 entries each, researchers focused on bottles, containers, tea bags, bags, and cups. MNPs were mostly analyzed in media simulating water while 212 entries referred to liquid and 121 to solid foodstuffs. The majority (580 entries) reported the detection of MNPs in the assessed medium. However, our critical appraisal of the study design and methods showed that many of these studies are of low quality. Of the 601 entries, only 50 were considered highly and 128 medium reliable.

Our systematic evidence mapping shows that under normal and intended use, plastic FCAs are a source of MNP migration that needs to be routinely and systematically assessed. It also points out that most published data are not reliable, indicating the need to integrate a standardized quality assessment approach in the study design. By publishing our data in an openly available database we hope to guide future research concerning study objects, methods, and ways of reporting. This will support safer plastic food packaging for critical applications.

#### **4.13.P-Mo403 Orally ingested microplastic affects a genes and associated signalling pathways in the pig's colon**

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Recently, there has been a growing interest in exploring the consequences of microplastics (MPs) consumption. One of the most common polymers used in food industry is polyethylene terephthalate (PET), whose effects on human and animal health have not been fully described. Recently, consumption of MPs has been linked to the onset of colorectal cancer. We hypothesize that *in vivo* exposure to PET affects various biological processes at the molecular level in the organs of the digestive system in immature piglets. To verify our hypothesis, we examined the effect of different doses of PET on changes in the global transcriptomic profile of distal colon.

The experiment was performed on 8-week-old immature gilts (n=15), divided into three groups receiving orally: 1) control – empty gelatin capsules; 2) low dose – 0.1 g/pig/day PET MPs in gelatin capsules; 3) high dose – 1 g/pig/day PET MPs in gelatin capsules for 4 weeks. Total RNA from distal colon tissues was isolated, and RNA-Seq analysis to identify differentially expressed genes (DEGs) on the NovaSeq 6000 Illumina platform was performed. Then g:Profiler enriched signaling pathways analysis was performed.

A low dose of PET revealed 251 DEGs (181 downregulated, 70 upregulated). The Gene Ontology (GO) annotation of biological processes (BP) indicated 26 terms, 4 ontology terms have been classified as molecular functions (MF) and 5 terms as cellular components (CC). KEGG, Reactome (REAC) and Human Phenotype Ontology (HP) databases revealed another 6 signaling pathways in which detected DEGs were engaged. DEGs were connected to e.g.: regulation of immune system processes (23 DEGs), biological regulation (138 DEGs), regulation of fatty acid metabolic process (6 DEGs). In turn, a high dose of PET revealed 358 DEGs (163 downregulated, 195 upregulated). The GO annotation of BP indicated 26 terms, 1 ontology term have been classified as MF and 3 terms as CC. KEGG, REAC and HP databases revealed another 7 signaling pathways in which detected DEGs were engaged. DEGs were connected to e.g.: lipid metabolic process (34 DEGs), retinol metabolism (6 DEGs) and cell differentiation and cellular developmental process (73 DEGs).

In this study, we identified for the first time signaling pathways that may be disrupted by microplastic ingestion, shedding light on the novel mechanisms that may lead to the adverse health effects of microplastic consumption.

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#### **4.13.P-Mo404 Development of Sampling and Analytical Methods for Measuring Microplastics with Sizes Greater than 20 µm in Tap Water**

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A novel sampling method with an analysis for microplastics (MPs) with sizes greater than 20 µm in tap water was developed to reveal their occurrence characteristics, such as their concentrations, polymer compositions and size distributions, with high precision. Micro-Fourier transform infrared spectroscopy followed by density separation could recover 91.0% of MP particles in tap water. Large amounts of water could make the standard deviations of detected MP concentrations much smaller, and the coefficient of variation decreased to 14.9% when the sampling volume was 1000 liter. The sampling method for MPs under faucets with unused plankton nets outside collected  $16.7 \pm 6.4$  particles by dry deposition from the atmosphere, but the coverage by lids could reduce the level of deposition. Finally, MPs in 1000 L of tap water originating from groundwater were analyzed. Twelve polymers were detected and their total concentrations ranged from  $78 \pm 10.4$  to  $2130 \pm 358$  particles  $m^{-3}$ . Polyethylene, alkyd resins and polymethyl methacrylate were dominant and their total concentrations contributed 56.5 to 94.0% to the total MP concentrations. The median sizes of MPs observed in all samples ranged from 30.1 to 60.4 µm and their dominant shape was of the fragment type. Tap water samples were also collected at 30 sites all around Japan. Tap water samples were also collected at 30 sites all around Japan to reveal occurrences of MPs in Japanese tap waters. Their concentrations ranged from 30 to 2110 pieces  $m^{-3}$ . Eleven polymers were detected in all samples, and polyethylene, polypropylene, polyethylene terephthalate, and polymethyl methacrylate were dominant. Principal component analysis of these data showed that their sources were polyethylene pipes, polypropylene pipes, lining fragments from pipes, and adhesive used to connect pipes.

#### **4.13.P-Mo405 International standardization of hazardous substance testing methods for recycled plastics for the Plastic Circular Economy**

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With the rapid growth of the electrical products, automotive and various plastic industries, the exposure and risk of micro & nano plastics is increasing, but the risks of various harmful substances to the human contained in plastic are being taken more seriously. Therefore, related international environmental regulations are being strengthened and research is being actively conducted. Accordingly, recent activities to reduce plastic usage and increase recycling of plastic waste to create a Plastic Circular Economy are highly active. However, as it is known that hundreds of types of hazardous substances can be detected in the human from recycled raw materials and products [1], actual industries want to confirm that exported/imported products do not contain hazardous substances and require qualitative/quantitative analysis of hazardous substances for QA/QC.

The need for standardization is also increasing on hazardous substances related to E&E, automotive, and various imported/export industrial products related to EU regulations (RoHS/ELV/REACH SVHC). WG 3 is taking the lead. Accordingly, WG 3 regularly conducts substance selection task activities to select candidates for future test method by survey. The recent candidate substances (Phthalates, PAHs, BPA, SCCP/MCCP) selected through this process were developed to the IEC 62321-series standard to develop test methods for the hazardous substances and conduct an IIS (international inter-laboratory study). Through the process of verifying test methods, we are developing horizontal standards that can be used around the world.

Recently, WG 3 are preparing to develop a test method for PFAS, which has been detected in various environments & human around the world and is of great interest and concern. WG 3 plan to develop an international standard by reflecting the requirement of various stakeholders regarding analysis methods & risk assessment that satisfy EU ECHA regulations. To achieve this, it is essential to secure samples with actual PFAS detected and RM/CRM, and the participation of global laboratories for future test method validation is necessary. Through this, WG 3 seeks to develop and provide to future core capabilities to verify whether recycled plastic raw materials contain hazardous substances, which are important in realizing the Plastic Circular Economy for the future society.

References: [1] Eric Carmona et al., Data in Brief, Volume 51, December 2023, 109740, doi.org/10.1016/j.dib.2023.109740

#### **4.13.P-Mo406 EFFECTS OF NANO- and MICROPLASTICS on THE HUMAN IMMUNE SYSTEM: HOW MUCH SHOULD WE WORRY?**

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Humans are broadly exposed to nano- and microplastics (NMPs). The main route of exposure is via alimentary intake, with an annual consumption of millions of particles. Once entered the human body, NMPs can spread via bloodstream, then supposedly reaching distant sites. To date, scarce information is available on the inflammatory responses, in particular on human primary cells. It is known that NMPs can induce cellular lysosomal toxicity, resulting in an increased expression of the pro-inflammatory nuclear factor NFkB, activated during stress responses and, in turn, the secretion of the pro-inflammatory cytokines interleukin(IL)8, IL1 $\beta$ , IL6 and tumor necrosis factor (TNF) $\alpha$ . In this context, we aimed at dissecting the effects of NMPs on human primary monocytes, cells endowed with a high phagocytic capacity and a pivotal role in the immune system.

First, we exposed freshly isolated human primary monocytes to plain fluorescent polystyrene spheres (diameter 10, 5, 1, 0.5 or 0.1 $\mu$ m). Results by microscopy and flow cytometry showed that NMPs were indeed internalized within monocytes, proportionally to the amount of NMP the cells were exposed to (ranging from 5000 to 5 $\mu$ g). NMPs exposure caused a sharp drop in cell vitality and viability primarily proportional to the amount of NMPs seeded, but also to the NMPs size. Then, a preliminary 27-target real-time PCR array, based on 18 cytokines, 4 chemokines and 5 growth factors, showed an overall pro-inflammatory profile elicited by NMPs, compared to the untreated control. Peculiarly, the smaller NMPs sizes induced a more pronounced inflammation, while the bigger ones were accompanied by a slightly higher transcription of the growth factors FGF and VEGF, together with the anti-inflammatory decoy receptor IL1ra. As the pro-inflammatory interleukine (IL)1 $\beta$  is directly linked to the activation of the inflammasome pathway, evaluation of ASC speckle formation. Indeed, an higher inflammasome activation was detected in particular with 0.5 and 0.1 $\mu$ m NMPs administration.

By these preliminary observations, we obtained a first insight into the pro-inflammatory effects of human primary monocytes exposure to NMPs. Our data point at what already presumed in the literature: the smaller it is, the worse it gets! Altogether, we aim at obtaining a detailed picture of the NMPs-elicited inflammatory response, to than be able to assess the putative detrimental contribution of NMPs to a number of clinical observation and inflammatory diseases.

#### **4.13.P-Mo407 Unraveling Photodegradation Mechanism of Surgical Masks from Microplastics and Nanoplastics Release**

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Since the Covid-19 pandemic, 129 billion masks have been consumed each month worldwide. Fate of masks not only include atmospheric, freshwater and seawater, but degraded masks may release micro(nano)plastics (MNPLs) that enter human body through inhalation and ingestion. Here, we simulated the photodegradation of surgical masks made from polypropylene in air, freshwater and seawater (equivalent to 71 days). Size-fractionated MNPL release was quantified using various vibrational spectroscopic imaging methods, and the morphology of nanoplastics were characterized with correlative microscopy. Macroplastics, microplastics, small microplastics and nanoplastics demonstrated various degree of sensitivity to degradation. Formation of hydroxyl and carbonyl groups were observed for microplastics > 10 µm in contrast to three layers of masks (macroplastic), indicating that MNPLs being a more sensitive indicator of photodegradation. Masks and microplastics exhibited a linear increase in crystallinity calculated from FTIR and Raman spectra, and a transition from ductile to brittle fibers that facilitated faster fragmentation into MNPLs. The total released microplastics and small microplastics was 30,696 and 7,864 particles per mask calculated from FTIR and Raman imaging data, respectively, with the most MNPLs released from the melt-blown middle layer as shown by cluster analysis. Nanoplastics with a minimum size of 66 nm were characterized correlatively by Raman imaging and scanning electron microscopy. This study quantified MNPL release and the alteration in physical and chemical properties after degradation, which have major implications for their environmental fate and human health risks.

#### **4.13.P-Mo408 Risk assessing micro- and nanoplastics for early-life human health: the AURORA Horizon 2020 research project**

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The scale of micro- and nanoplastic (MNP) pollution is becoming increasingly clear yet little is known about how this pollution impacts human health, particularly during early-life stages. The AURORA project will deliver an actionable European roadmap for early-life health risk assessment of MNPs to support regulation of MNPs, the products and processes that generate secondary MNPs, and development of safer alternatives. Within the project, we are focusing on MNP exposures and toxicological and health effects during pregnancy, in utero, and in early life. MNPs have been shown to cross the placental barrier *in vitro* and *in vivo*, underlying the urgent need to understand the impact of MNPs on reproductive and early-life health. AURORA will advance this understanding by significantly enhancing exposure assessment capabilities for measuring MNPs and MNP-associated chemicals (e.g. additives) in tissues relevant for early-life development (placenta, cord blood, amniotic fluid, meconium, fetal tissue). AURORA adapts and integrated approach by combining in-depth characterization methods (microscopy and spectroscopy) and scalable methods (mass-spectrometry) to develop strategies for both detailed and large-scale toxicological characterization, exposure assessment, and epidemiological studies. This will be combined with a novel tiered-testing approach and epidemiological investigations to provide the first extensive evaluation of maternal and fetal MNP exposures and health perturbations, including placental function, immune-inflammatory responses, oxidative stress, accelerated aging, endocrine disruption, and child development. While developing and applying the tools and methodological workflows of the AURORA research program, we are creating a risk assessment framework specific to MNPs and identifying the remaining knowledge gaps and priorities needed for comprehensively evaluating the impact of MNPs on early-life health. The AURORA project started on April 1, 2020 and is part of the EU Horizon 2020 funded research CUSP cluster. The project is a collaboration between 11 partners based in Europe and the US, and it will run for 5 years. A central focus of AURORA is effective dissemination of the research findings, and for this reason we will closely collaborate with different stakeholders from academia, policy arenas, civil society and industry.

#### **4.13.P-Mo409 Nanoplastic type influences their biological effects on brain microglia**

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As plastic pollution continues to grow in various ecosystems, potential harmful effects of micro/nanoplastics have become a great concern. So far, the biological effects of nanoplastics were investigated by mainly using polystyrene as a model plastic type. However, a majority of environmental plastic wastes is the mixture of various types of plastics, such as polypropylene (PP), polyethylene (PE), polystyrene (PS) and polymethylmethacrylate (PMMA) and so on, in real ecosystem. In this study, we investigated the biological effects of nanoplastics with the similar size on microglia functions using three different types of plastics (PS, PP and PMMA). In our cultured primary rat microglia, three different nanoplastics were rapidly internalized into the cell within 3 hours by confirming fluorescent imaging. Among the groups with these nanoplastics, the group exposed to PMMA nanoplastics showed the highest morphological change and expression of the markers for microglia activation. In addition, we found that PMMA nanoplastics increased the migration ability of microglia by inducing the expression of chemokines, such as CXCL1 and 2. These data demonstrate the possibility that the nanoplastics exposed microglia may accelerate neurological disorders by enhancing the recruitment of microglia and peripheral immune cells across the blood brain barrier in neuropathological conditions.

#### **4.13.P-Mo410 Extended application of physiologically based pharmacokinetic: developing particle- and species-related parameters**

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Distribution of particles in the human body is associated with the nanotoxicity. Physiologically based pharmacokinetic (PBPK) tools can simulate the distribution and elimination of NPs but are primarily dependent on experimental data. PBPK tools require parameters related to properties of various particles and species. However, it is difficult to quantify the effect of particle properties on particle behavior *in vivo*. The derivation of PBPK models from mice to humans is also necessary for human risk assessment.

In this study, we developed a scope on extending the application of PBPK models to various particles and species, estimating particle-related and body/species-related parameters involved in PBPK models. Previous results show that the phagocytosis rate and clearance rate constants of nanoparticles from blood can be estimated by simulating interaction between nanoparticles and tissues. Generic allometries of species-related parameters can estimate physiological data for different species. Our results proved the allometries of organ blood flow and metabolic rates for high-perfusion organs are organ weight-dependent. We expect the methods for deriving particle- and species-related parameters can extend the application of PBPK models to various nano/micro plastics and species.

#### **4.13.P-Mo411 Polypropylene nanoplastics enhances the intestinal inflammation induced by disinfectants in zebrafish larvae**

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Microplastics are ingested through various routes, including drinking water, food packaging, and food itself. Previous study suggests that ingestion of microplastics can lead to oxidative stress, intestinal inflammation, and alterations in the gut microbiota composition. However, most research has focused on healthy conditions that do not accurately represent the real-life situations of individuals undergoing intestinal inflammation due to various factors such as dietary choices, drugs, and environmental pollutants. Therefore, our research aims to design experimental conditions that are in line with the health status of individuals experiencing intestinal inflammation. Here, we used benzalkonium chloride (BAC) as a representative disinfectant which could induce intestinal inflammation. BAC is a well-known environmental pollutant which ingested into the intestine via drinking water and personal health care products, including mouthwash. Additionally, polypropylene nanoplastics (PPNPs) were used as a microplastic model which was ingested into the gut via drinking water, food, and mask. In this study, zebrafish (*Danio rerio*) was used as an animal model to detect intestinal toxicity between BAC and PPNPs. Zebrafish larvae were exposed to BAC at 3 days post fertilization (dpf) for 48 h to induce inflammation in the gut. Then, larvae were exposed to PPNPs at 5 dpf for 48 h by ingestion. Our results revealed that PPNPs enhanced toxic effects in the inflamed intestine induced by BAC. PPNPs with pre-exposure of BAC were more 2.8 times accumulated in the intestine than that with single-exposure of PPNPs. PPNPs was mainly distributed in the intestine but did not induce the migration of immune cell. However, in the inflamed intestine by BAC, PPNPs induced 3 times the macrophage activity and also increased expression of inflammatory responses such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-8. In this result, although PPNPs did not induce severe intestinal inflammation in zebrafish larvae, they enhanced toxic effects in the weak gut by BAC. The findings show to better understanding of the potential risks of microplastic by ingestion in terms of multiple toxicity in human health.

#### **4.13.P-Mo412 Micro and Nanoplastics (MNPs) as Potential Hazards for Human Health: Effects of Polystyrene NPs, Polyethylene MNPs and Fishing Net Leachates on Human Intestinal Caco-2 Cells**

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Plastic pollution has increased exponentially in recent years, and both aquatic and terrestrial ecosystems are affected by their presence. Small-sized plastics, known as microplastics (MPs, 1 to 1000  $\mu\text{m}$ ) and nanoplastics (NPs, 1 to 1000 nm), stand out for their ubiquity and potential interactions with living organisms. Among plastic polymers, polystyrene (PS) and polyethylene (PE) are two of the most widely used thermoplastic polymers in food packaging in Europe. Fishing nets contribute to MNP pollution in the marine environment and are made of different polymers including polyamide (PA) and additives. The objective of the present work was to carry out a hazard assessment of commercial MNPs and fishing net leachates containing MNPs using a human intestinal cell line *in vitro*. For this, Caco-2 cells were exposed to different concentrations of commercially available PS NPs (50, 500 and 1000 nm) and PE MNPs (200-9900 nm) and to polyamide 6 (PA 6) and 50% bio-based polybutylene succinate (PBS) fishing net leachates containing MNPs. The behavior of PS and PE MNPs and leachates in cell culture medium was characterized by Dynamic Light Scattering (DLS) and cell viability was assessed by the MTT assay after 24 hours exposure. The results obtained by DLS showed that particles had a negative surface charge and high polydispersity, and that most of them tended to aggregate in cell culture medium. According to the results obtained *in vitro*, commercial pristine PS and PE MNPs did not show any cytotoxicity towards Caco-2 cells, whereas PA 6 and PBS fishing net leachates significantly reduced cell viability after 24 hours exposure, possibly as a result of released additives present in plastic formulations. Our results suggest that the next steps of research into MNPs hazardous effects should be focused on testing environmentally relevant MNPs and plastic additives on human cell models, as they may produce higher toxicity than pristine MNPs. \*Funded by the Basque Government project MIKRONANOPLAS (PA22/01) and grant to consolidated research groups

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#### **4.13.P-Mo413 Physiologically Based Kinetic (PBK) Modelling for Human Exposure to Micro- and Nanoplastics**

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Microplastics (plastic particles < 5 mm, including nanoplastics < 1 µm) have become a global concern. Humans are exposed to microplastics via inhalation, food consumption and dermal contact, potentially adversely affecting human health.

Physiologically based kinetic (PBK) models are valuable tools for understanding the internalisation of chemicals. PBK models provide chemical concentration estimates over time in specific tissues based on descriptions of absorption, distribution, metabolism, and excretion (ADME) processes and the physiology and anatomy of the organism. However, human PBK models for microplastics are currently lacking. Therefore, the present study integrates the existing information on relevant absorption, distribution and excretion pathways of microplastics in the human body and develops a conceptual PBK model for human exposure to microplastics.

An extensive literature search was conducted regarding relevant ADME processes of microplastics in humans. Only ten studies on *in vivo* and *in vitro* mammal models were identified as relevant. As nanoplastics are expected to penetrate the organism sharing similar physiochemical characteristics with nanoparticles (e.g. inert, nano-sized), information on engineered nanoparticles from nanomedicine and nanotoxicology was also included.

Absorption largely depends on particle size, where particles < 10 µm are most likely to penetrate the gastrointestinal barrier and particles < 1 µm could cross the alveolar wall. The distribution of microplastics between tissue and blood is governed by permeability and surface energy. The reticuloendothelial system plays a major role in taking up microplastics in the liver, spleen and lungs. We present the conceptual PBK model with mass balance equations and required input parameters which can be potentially derived from the collected data. We propose combining mechanistic (estimating input parameters as functions of organisms' and particles' properties) and probabilistic approaches (e.g. the Bayesian method) to estimate parameter values to expand the applicability domain of PBK models to diverse microplastics. Future work includes evaluating the model using data from microdosing and epidemiological studies, examining variations from cell cultures and animal models with varying conditions, and understanding the role of biomolecular corona in the uptake and biodistribution of microplastics.

#### **4.13.P-Mo414 Impact of Polystyrene Nanoparticles on Lung Epithelial Barrier Formation and Functionality**

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Micro- and nanoplastics (MNPs) are one of the most widespread pollutants of our time. They can be manufactured in specific size for a specific purpose or be released into the environment through the degradation of plastic products. Humans are generally exposed to MNPs via two main routes: food ingestion or inhalation of air. In the latter case, it has already been reported that airborne particles can enter and accumulate in the lower respiratory tract, leading to occupational diseases in textile workers. The aim of this study is to assess the impact of 300 nm europium doped polystyrene (PS-Eu) particles on the integrity and functionality of the lung barrier, especially in terms of surfactant production. In this context, the use of europium doped particles is instrumental to study particle translocation and uptake as europium is a fluorescent heavy metal that can be detected via different imaging techniques. After characterizing our particles in detail, we performed standard cytotoxicity assays on A549 cells grown under submerged conditions and found that PS particles can affect the activity of acidic organelles in a dose-dependent manner using the neutral red uptake assay. In contrast, no effects of PS-Eu on viability were detected using lactate dehydrogenase (LDH) or resazurin assay, although particle uptake was confirmed by TEM. We then examined the effects of PS-Eu on the same cells grown either in air-liquid interface (ALI) or submerged conditions using transwells. No clear effects on epithelial permeability or electrical resistance were detected by Lucifer Yellow assay or TEER measurement. Similarly, these particles did not appear to induce cell death or translocate towards the barrier compared to controls. However, a dose-dependent decrease in surface tension associated with increased mitochondrial activity was observed in both types of culture systems. Overall, this could indicate that the nanoplastics may boost, upon uptake, surfactant production and influence vesicle acidification (which are both ATP-demanding processes) ultimately leading to an increase in mitochondrial respiration to meet the new energetic requirements. Future analysis aiming to study the production and recycling of surfactants by ELISA and TEM could shed more light on the molecular mechanisms triggered by these particles.

#### **4.13.P-Mo415 Correlative Spectroscopy and Microscopy Analysis of Micro- and Nanoplastics in Complex Biological Matrices**

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Micro- and nanoplastic (MNP) particles are widespread environmental pollutants, with recent studies detecting their presence in various human tissues and bodily fluids. However, the precise detection and quantification of MNPs in biological samples, particularly nanoplastics, face challenges due to biological backgrounds and variations in sample preparation, measurement settings, and data evaluation protocols. Here, we developed novel analytical workflows based on context application of cutting-edge spectroscopy and microscopy techniques supported by Machine Learning (ML) data evaluation to enable identification and differentiation of MNPs in intricate biological contexts, associated with human interaction. We utilized optical and electron microscopy methods for high-resolution imaging at various scales, complemented by spectroscopy techniques for definitive MNP identification based on their chemical fingerprint. In-vitro exposure of human lung Calu-3 cells to polystyrene (PS) particles (<2 µm in diameter) in a submerged setting resulted in cell adhesion and ingestion, as confirmed by Scanning Electron Microscopy (SEM) and 3D Stimulated Raman Scattering (SRS) imaging. The existence of PS particles both on the cell surface and within cells hinders nutrient absorption, leading to cytotoxic effects, as evidenced by cell viability assays, particularly at a PS concentration above 1 mg/ml. Adaptive ML algorithms applied to SRS images facilitated MNP localization, counting, and categorization by size and shape. Zebrafish and mouse in-vivo exposure to PS particles through drinking led to organ accumulation, with zebrafish taking up 50 nm PS particles via digestion, as measured by Fluorescence (FL) and SRS. Mice showed 250 nm PS particles in various organs including brain, indicating blood-brain barrier crossing and accumulation at lipid-rich regions, as shown by Second Harmonic Generation (SHG), SRS, and Coherent Anti-Stokes Raman Scattering (CARS). This study demonstrates how correlative molecular and microscopy imaging can be employed to detect MNPs in complex biological matrices and understand their effects and fate, focusing on cytotoxicity, disruption of nutrient uptake, and organ accumulation. These findings reveal MNPs' translocation across cells, tissues, and organs, being relevant to human exposure through inhalation and ingestion and providing grounded risk assessment.

#### **4.13.P-Mo416 True-to-life Nanomaterials for Evaluating Nanoplastics Impacts on Human Health**

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Environmental nanoplastic pollution is a great issue affecting all ecosystems. The need to understand the possible implications of nanoplastic pollution on the environment and human health is becoming increasingly pressing. Given the complexity of separating nanoplastics from environmental samples, studies have been so far conducted using synthetic polystyrene nanobeads (NBs). There is an urgent need to create nanomaterials that better reflect the real characteristics of nanoplastics naturally formed, viz. true-to-life nanoplastics (T2LNPs), to close the gap between the laboratory parameters and the rules of nature, and to provide more realistic understandings of the characteristics of nanoplastics.

Here, we present a study on the production and characterization of T2LNPs from daily-used plastics, the investigation of bio-interfaces through the study of protein corona formation on T2LNPs with respect to synthetic NBs, and the assessment of their toxicity of human cells. T2LNPs samples were produced from daily life plastic items subjected to mechanical fragmentation through an ultracentrifugal mill operating in cryogenic conditions. The produced T2LNPs were characterized by Fourier transform Infrared spectroscopy to investigate their chemical nature and check the absence of induced chemical modifications. Morphology and size distribution analyses were performed through Atomic Force Microscope and Scanning Electron Microscopy. Finally, the protein corona formation from human plasma on T2LNPs and NBs was examined by electrophoresis and *in-vitro* toxicity at different exposure time and concentrations was assessed through MTT assay.

The differences detected in the protein corona profiles of T2LNPs and NBs confirm the gap between controlled models and the complexity in real-life scenarios, supporting the need to develop true-to-life materials as reasonable models for environmental nanoplastics. The broad heterogeneity in size and shape shown by fragmented T2LNPs gives the nanomaterial a peculiar and different behavior compared to the defined pristine nature of NBs, nominating T2LNPs as a more faithful material for naturally occurring nanoplastics and opening the possibility to new and unexpected results in biological interactions, as suggested by the preliminary results of *in-vitro* toxicity tests.

#### **4.13.P-Mo417 Human oral exposure to nano- and microplastic polymers and plastic additives in a cross-sectional population-based study of Barcelona (Spain)**

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**BACKGROUND:** Micro and nanoplastics (MNPs) are emerging contaminants found in food, drinking water, and air. Humans are continuously exposed to MNPs through ingestion and inhalation. However, the magnitude of the exposure in the population and the associated potential health effects remain unknown. **OBJECTIVES:** We characterized human exposure to MNPs and plastic additives through drinking water and food in the general adult population of Barcelona, Spain. **METHODS:** The study population includes 50 healthy adults of the Metropolitan Area of Barcelona. Personal information on diet, socio-demographics, lifestyle, *etc.* has been ascertained through questionnaires. We conducted home visits to collect tap water samples (5 liters). Food consumed during 4 days was collected in a subset (N=10). Water samples were first filtered through a stainless-steel sieve with a 20 µm mesh, followed through a 0.70 µm glass microfiber filter. An ultrasonic-assisted extraction was performed to extract MNPs with 10 mL of toluene for 10 minutes. For MNPs analyses, a double suspect screening

approach based on size exclusion liquid chromatography combined with high-resolution mass spectrometry (LC(SEC)-HRMS) was employed. Plastic additive analyses were carried out based on high-performance liquid chromatography coupled to HRMS. Statistical analysis included a univariate description of variables. **RESULTS:** A total of 49 tap water samples were analyzed, with 26% of samples containing polyethylene (median 1016.6 ng/L; range 26,75 – 8114,0). Polyisoprene was detected in 1 sample (0,63 ng/L). All tap water samples revealed the presence of 9 to 10 different plastic additives above detection limits, with varying quantification rates. Dipropylene glycol dimethyl ether was quantified in 44 (90%) samples, tributyl phosphate in 28 (57%), triethyl phosphate in 22 (45%), lauro lactam in 21 (43%), diethyl phthalate in 19 (39%), diacetone acrylamide in 3 (6%), hexamethylcyclo-trisiloxane in 1 (2%), and triphenyl phosphate, tris (2-butoxyethyl) phosphate, and dibutyl phthalate in none. Diethyl phthalate displayed the highest concentrations (median 5.91 ng/L). When compared to previous studies in Barcelona, we observed lower occurrence of polymers, suggesting temporal variability. Results for food items will be available for the time of the conference. **IMPACT:** This project generates novel scientific evidence on the extent of exposure to MNPs as a basis for further health risk assessments.

#### **4.13.P-Mo418 Cytotoxic And Genotoxic Effects Of Nanopolystyrene And Bisphenol A Per Se And In Combined Exposure In A Gastric Cell Line – How High Is The Current Exposure Risk?**

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Currently, due to the massive use of plastics worldwide, it is inevitable that nanoplastics (NPs) and its additives such as plasticizers, including Bisphenol A (BPA), enter in the body as single substances or/and as mixtures. NPs present a high hazardous potential given the evidence of toxic effects on a cellular level and the levels of plastic oral exposure via ingestion and absorption of these substances in gastric tissues. Furthermore, the ability of acting as a vector for other pollutants and its ability to be internalized by cells potentiate long term effects. Therefore, in order to efficiently assess the risk for human health, it is crucial to evaluate the exposure response in complex and realistic scenarios.

The aim of this *in vitro* study was to investigate the cytotoxic and genotoxic effects of polystyrene (PS)-NPs and BPA through individual and combined exposures on the gastric GP202 cell line. Environmental relevant concentrations previously tested in literature were selected, for PS-NPs - 20, 100 and 200 µg/mL and for BPA - 0.1, 1 ng/mL, and 0.04 ng/mL as the new reference value proposed by EFSA. Cytotoxicity was assessed by Cell Titer- Blue® assay and genotoxicity by cytokinesis-block micronucleus assay, following the 487 OECD Guideline. Colchicine (5µg/mL) was used as positive control.

Our results indicate no cytotoxicity associated to either individual or combined exposures of NPs and BPA. Regarding to genotoxicity it was found that all the endpoints – micronucleus, nucleoplasmic bridges and nuclear buds – were significantly increased in the worst case-scenario of combined exposure (200 µg/mL PS NPs + 1 ng/mL BPA) (69±6.15, 26±2.73 and 37±3.15) in comparison with negative control (40±6.56, 11±1.53 and 4.0±0.58), respectively. These results highlight the importance of further studies with combined exposures of NPs and BPA in order to develop, carry out and evaluate the safety risks of chemical mixtures.

#### **4.13.P-Mo419 Influence of in vitro Gastrointestinal Digestion and UV-Aging of Tire Rubber Microplastics on the Release of Chemical Additives and Toxicity Assessment**

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Microplastics (MPs) contain a broad variety of additives, including antioxidants or plasticizers, along with unreacted monomers and sorbed contaminants. Traffic abrasives from tires and crumb granulates from discarded tires have emerged as significant contributors to MP pollution. Concerns have often arisen about the potential for toxic compounds to leach from MPs, yet implications for human health are not well understood.

In this study, we investigated the release of potentially toxic chemicals from three types of polydisperse MPs: tire rubber (TR), polyamide-6 (PA-6), and thermoplastic polyurethane (TPU). The effects of *in vitro* digestion in human gastrointestinal fluid simulants and UV-aging of the MPs on the chemical leaching was investigated. Leached chemicals were analyzed using gas chromatography-mass spectrometry. Released metal contents were quantified by microwave-assisted extraction followed by inductively coupled plasma-mass spectrometry. All MPs were characterized for their cellular effects, specifically for cell viability and cytochrome P450 1A1 (CYP1A1) induction in human intestinal CaCo-2 cells.

Most chemicals were leached from TR particles. In particular, benzothiazole (~170 µg/g), (3H)-benzothiazolone (~168 µg/g) and zinc (~1150 µg/g) were quantified in high concentrations in small and large intestine fluid simulants. Cell viability did not decrease within 24 h of exposure. TR particles induced CYP1A1, but this effect decreased after UV-aging. Benzothiazole derivatives as well as diethyl and dibutyl phthalates were identified as important contributors to CYP1A1 induction. Selected leached additives were further investigated for potential toxic effects by utilizing the EPA ToxCast database, revealing links of



20 leached compounds to specific adverse outcome pathways. Our findings indicate that the release of specific additives, particularly from TR particles, might pose a risk to human health and that *in vitro* digestion and UV-aging modulated the leaching of these additives.

#### **4.13.P-Mo421 Washable vs Disposable Face Masks: Million microfiber releases and their toxicities**

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The widespread use of face masks has raised environmental concerns related to microplastic fibers. While various studies have explored the release of microfibers from disposable masks in controlled environments, their release in real-life conditions has been understudied. Similarly, the release of microfibers from washable masks has received limited attention. In this study, we comprehensively investigated the release and toxicity of microfibers from disposable and washable masks in simulated aquatic environments and real-life scenarios (disinfection, hand washing, and machine washing). We quantified microfiber release using Nile red visualization and identified the polymer composition by  $\mu$ -Raman spectrometry. Our findings revealed that disposable masks released microfibers ranging from 18 to 3042 microfiber/piece, whereas washable masks released  $6.1 \times 10^4 - 6.7 \times 10^6$  microfibers/piece. Hand and machine washing released a higher number of microfibers among all simulated conditions. Notably, a negative correlation was observed between microfiber release and the proportion of embossing on the surface of disposable masks. Microfibers from disposable masks primarily comprised polypropylene (PP), while washable masks predominantly released polyethylene terephthalate (PET) and cellulose microfibers. Furthermore, our results indicated that PP microfibers affected the swimming behavior of zebrafish, while PET microfibers hindered early-stage zebrafish hatching. This study offers new insights into the source of microplastic fibers in realistic settings and contributes to our understanding of the environmental impacts of discarded face masks and microfibers, with implications for both environmental and human health.

**Keywords:** Single-use masks, Reusable masks, Microplastics, Microfiber shedding, Microfiber release, Biological impacts, Toxicity, Zebrafish.

#### **4.13.P-Mo422 Pro-inflammatory responses in cells induced by nanoplastic exposure**

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The increasing global production of plastic items has led to a critical pollution issue, namely due to their improper disposal in the environment, especially in marine ecosystems. Polystyrene, one of the most produced polymers in 2021, finds extensive use in packaging, insulation, electronics, and construction. The exposure of this material to environmental factors facilitates fragmentation into smaller particles, namely into nanoplastics (NPs), posing substantial threats to both animal and human health.

NPs has the capacity to traverse biological membranes, are easily ingested and have a great potential to bioaccumulate in organisms. Internalization of NPs is associated with oxidative stress and tissue damage, with potential disruptions to membrane receptors and activation of inflammatory signaling cascades, inducing inflammation and several adverse outcomes. However, the underlying mechanisms of these effects are poorly understood. This study hypothesized that NPs induce inflammatory signaling through the NF- $\kappa$ B and/or p38 MAPK pathways. To test this hypothesis, human colorectal adenocarcinoma cells (HT29) and mouse microglia cells (N9) were exposed to different concentrations and sizes of polystyrene NPs. Cell viability, protein expression levels of p50 NF- $\kappa$ B subunit, p38, and TLR4, as well as pro-inflammatory gene transcription, namely IL-1 $\beta$ , iNOS and TNF $\alpha$  were assessed.

Results indicated that NPs did not significantly impact HT29 cell viability but markedly reduced N9 cell viability, especially with smaller-sized particles. Nuclear translocation of p50 NF- $\kappa$ B and p38 was observed in HT29 cells, suggesting inflammatory responses. However, N9 cells exhibited no significant variations in translocation. Pro-inflammatory gene expression showed increased IL-1 $\beta$  in both cells and iNOS in N9 cells, with TLR4 and p38 inhibitors attenuating these responses.

In conclusion, this research highlights the inflammatory effects of NPS, with variability depending on cell type and signalling pathways. TLR4 appears to play a central role, and further investigation is needed to elucidate the involvement of additional factors such as p38. The study underscores the potential disruption of tissue homeostasis by nanoplastics, emphasizing the need for continued research to understand their implications for inflammation and human health.

#### **4.13.P-Mo423 Combined effects of polyethylene nanoplastics and mercury on viability and migration capacity of human cell lines**

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The accumulation of plastic waste and the widespread presence of its degradation products, micro- and nanoplastics, demand an urgent evaluation of their potential health risks. Nanoplastics (NPLs) have a negative impact on human health and the environment due to their high persistence and high level of fragmentation. In the environment, NPLs coexist and interact with pre-existing contaminants, leading to biological effects (bioaccumulation and/or toxicity) that are poorly understood.

Metal(loids) have a particular affinity for nanoadsorbents, and considering their persistent and toxic nature it is imperative to assess the toxic profile resulting from these interactions. Therefore, this work assesses and compares the joint effects of two relevant water contaminants: polyethylene nanoplastics (PENPLs) and mercury (Hg), on the major routes of human exposure: inhalation and ingestion. For that, A549, HepG2 cells were exposed to single and binary mixtures of PENPLs at 6.25 – 400 µg/mL and Hg 0.5 - 40 mg/L. For binary mixtures, two concentrations were selected based on the results of single exposure: (1) no observed effect concentration and (2) lowest observed effect concentration. Viability was assessed by MTT after 24 hours exposure and the ability of a single cell to grow into a colony was assessed by the colony formation assay after 7 days exposure. Meanwhile, cell migration, a biological process that is critical to a variety of physiological functions such as inflammatory responses, was assessed by wound healing assay at 0, 6, 24h. Our results showed that PENPLs can induce cytotoxicity to human cell lines after 24h for concentrations above 50 µg/mL and for prolonged exposure (7days) cytotoxicity was visible for lower concentrations (6.25 µg/mL). Migratory capacity was decreased after 24h exposure, in a concentration-dependent manner. The relative migration rate of A549 and HepG2 cells treated with only PENPLs was 25% and 11% at 24h, respectively. We hypothesised that i) PENPLs significantly enhance Hg toxicity; ii) PENPLs co-exposure with Hg will affect each other's individual toxicity, either by antagonism or synergy; iii) migratory capacity will be affected differently in cells exposed to binary mixtures. The results of this study will improve our understanding of how NPLs affect human health and provide insights into the complex interactions between NPLs and metals, contributing to a comprehensive assessment of their combined toxicity in humans.

#### **4.13.P-Mo424 Developing Human Noncancer and Reproductive/Developmental Effect Factors for Nano- and Microplastics in LCA**

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Background: Nano- and microplastics (NMPs) are ubiquitous in our environment (air, water, food chain) and their presence is believed to be responsible for numerous effects on ecosystems and human health. However, toxicological assessment of potential impacts of NMPs on human health is still lacking in life cycle impact assessment (LCIA), and the detection of these microparticles in human bodies has made it crucial to better understand and characterise such potential impacts.

Objective: The aim of this research is to develop human noncancer and reproductive/developmental effect factors for NMPs in LCIA as part of the MarILCA project, based on the study of *in vivo* animal data and the latest framework in human health impact assessment

Methods: Experimental studies concerning *in vivo* mammal toxicity, and in which a critical effect was observed through daily oral ingestion of NMPs (virgin or with additives) were selected. The obtained curated database was then processed to generate a representative chronic human benchmark dose (BMDh), later extrapolated into an effect factor for LCIA.

Results: A preliminary regulatory BMDh for general noncancer effects of polystyrene NMP changes from  $1.06 \cdot 10^{-2}$  (all recovered data) to  $2.07 \cdot 10^{-2}$  mg/(kg.d) when considering only true adverse data (when an experiment). The associated effect factors are 44.0 and 22.7 DALY/kg for all and real adverse data respectively. Concerning reproductive/ development effects, BMD\_h were found of the same order of magnitude ( $2.13 \cdot 10^{-2}$  to  $3.21 \cdot 10^{-1}$  mg/(kg.d)) with a calculated effect factor of 404.3 or 26.8 DALY/kg for all and real adverse data respectively.

Discussion: Polystyrene NMP has been allegedly found among the most toxic chemicals. This research calls for a larger discussion and deeper analysis of the obtained results to (in)validate them, as the findings are concerning. Future research into NMPs is needed to improve our understanding of their mode of action and factors affecting their potential effects.

#### **4.13.P-Mo425 Production and Testing of Micro- and Nano-PET Materials for Human Digestion and Enzymatic Hydrolysis Studies**

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MNP research is a rapidly growing field, but it is hampered by the lack of relevant, harmonised analytical techniques as well as test and reference materials with a variety of controlled properties, which are essential for representative, accurate and reproducible results. The aim of this work is to present current options for laboratory production of micro- and nano-sized particles of polyethylene terephthalate (PET), one of the most common polymers that has been detected, among other matrices, in beverages and human feces, suggesting an active interaction with the human digestive system. Irregular PET MPs obtained by a mechanical top-down method were subjected to simulated static gastrointestinal digestion (INFOGEST method) and dynamic colonic fermentation (simgi®) *in vitro* to investigate the mechanism and effects of this interaction that may potentially lead human health risks; the microPET suffered biotransformations and altered the microbiota. Spherical PET NPs, obtained by dissolution and subsequent precipitation, were submitted to enzymatic hydrolysis by a recently engineered protein nanopore (npFraC<sub>m1</sub>) to evaluate innovative end-of-life biotechnology-enabled strategies for PET re- or up-cycling that may minimize the plastic debris.  $\mu$ -Raman spectroscopy, a powerful technique to characterise filtered samples in terms of polymer type as well as particle number, shape and size distribution up to sub-micron sizes, is used in this work to monitor both processes as well as the synthesis, to obtain relevant structural information from the MNPs. The results highlight the pivotal role of advanced analytical techniques and tests materials in unraveling the intricacies of polymer degradation pathways and effects.

#### 4.13.P-Mo426 Chronic Effects of Nano and Microplastics on Reproduction and Development of Marine Copepod *Tigriopus Japonicus*

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This study aimed to examine the impact of chronic (30 days) exposure to polystyrene microplastics (PS-MPs) of different sizes (50 nm and 2  $\mu$ m) and at different concentrations (0.5  $\mu$ g/L and 100 mg/L) to marine copepod *Tigriopus japonicus*. Polystyrene microplastics affected survival rates in size- and concentration-dependent manners. The LC<sub>50s</sub> values of 50 nm and 2  $\mu$ m PS-MPs were 0.10 mg/L and 3.92 mg/L, respectively. The developmental time was delayed by 50 nm PS-MPs, and *Usp* expression was downregulated. Reproduction was negatively affected by 2  $\mu$ m PS-MPs even at environmentally relevant concentrations; however, the expression of *Vtg* was not altered. The production rates of reactive oxygen species and nitric oxide also increased after exposure to PS-MPs; but this effect was independent of particle size. The expression levels of *Cat* and *Tnf*, genes related to oxidative stress and inflammation, respectively, were upregulated by exposure to PS-MPs, independently of particle size. Meanwhile, the level of oxidative stress in *T. japonicus* was not significantly affected by PS-MPs at environmentally relevant concentrations. This study suggests that nano-sized PS-MPs are not always more toxic than micro-sized PS-MPs, and that oxidative stress is a key factor in determining the toxic effect on *T. japonicus* at high concentrations.

#### 4.13.P-Mo427 In Vitro Cell Transforming Capacity of 3D Printed Objects-Derived Plastic Particles

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Humans are continuously exposed to micro- and nanoplastics (MNPs) generated from the fragmentation of larger waste plastics. MNPs can accumulate in tissues and organs, raising concerns on their potential effects on human health, especially the induction of cancer. Opposite to the expensive and ethically concerned carcinogenesis studies in rodents, *in vitro* cell transformation assays (CTA), as the validated Bhas-42 CTA, allow the *in vitro* simulation of the *in vivo* initiation and promotion stages of carcinogenesis.

The aim of the present study was to assess the cell transforming capacity of MNPs generated during breaking down and degradation of 3D printed objects at the end of the lifecycle. For that purpose, different materials were generated by cryomilling 3D objects printed using four different types of plastic fibers: polycarbonate without and with single walled carbon nanotubes (PC and PC-CNT, respectively), and polypropylene without and with silver nanoparticles (PP and PP-Ag, respectively). Then, the materials were sieved to obtain particles below 5  $\mu$ m, which were characterized and dispersed in water according to the NanoGenotox protocol. The cell transformation and concurrent cell growth assays were performed as recommended by the OECD guidance 231. Bhas 42 cells were treated with each type of particles (6.25 – 100  $\mu$ g/mL) either from day 1 to 4, or from day 4 to 14, for the initiation and promotion assays, respectively, and the number of transformed foci was scored on day 21. Parallel cultures were set to assess cell growth, cellular internalization and the expression of genes involved in cell proliferation and adhesion on day 7.

Under the exposure conditions of the initiation assay, a dose-dependent decrease in cell growth was observed for all the materials, and it was especially pronounced for PP-Ag particles. On the other hand, a slighter effect was observed under the promotion conditions. None of the particles significantly increased the number of transformed foci in the initiation or promotion assays, despite having been efficiently internalized by the Bhas 42 cells. Gene expression analyses are in progress.

In conclusion, none of the 3D printed objects-derived MNPs analyzed in the present study seem to induce the transformation of Bhas-42 cells either by genotoxic or non-genotoxic modes of action.

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#### **4.13.P-Mo428 The Fate and Effects of Micro and Nanoplastics in the Human Body – Insights from the EU-funded PlasticsFatE Project**

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In the environment, plastics degrade slowly to micro-plastics (MP) (< 5 mm) and nano-plastics (NP) (< 1 µm). These can consist of a complex mixture of polymers and associated chemicals (AC) added to improve performance or adsorbed from the environment. There is strong evidence that humans are exposed to MNP through diet, drinking water or inhalation. However, our understanding of the extent of human exposure to MNP is poor. One challenge in assessing the impact of MNP and AC on human health is ensuring our measurement and testing methods are capable of producing reliable data. Despite progress in measuring plastic particles in environmental media, reliable measurements and toxicity testing of these particles in the human body, in food, drinking water or air is lacking, even more so for NP that may enter human cells. This hampers the development of standards and test guidelines for science-based risk assessment and the implementation of relevant policies and regulations.

We have established a test materials strategy, using a range of polymers that humans are routinely exposed to, to address these issues. These materials have been produced by established physicochemical and mechanical techniques and characterised by relevant measurement methods. We have further developed methods for measuring MNPs in complex matrices, such as food, beverages and human tissues. This has provided us with a solid foundation on which to assess these materials in a variety of *in vitro* and *in vivo* model systems, that mimic normal exposure routes for humans (mainly inhalation and ingestion). Importantly, these well-characterised materials allow comparison and read across of different experiments.

Most of the common plastics have very limited effects on biological systems when in pure or virgin form. However, many contain additives when processed into final products, can change their physicochemical properties as they age and can adsorb contaminants from the environment during their use phase. These additional chemical and biological materials can be carried across cellular barriers, leading to inflammatory and cytotoxicity responses. We have also observed bacterial colonisation of microplastics and gene transfer between bacteria on microplastics and tyre wear particles.

This knowledge has been applied in a series of occupational and environmental case studies to investigate exposure and biological responses to MNPs of workers at plastic packaging, production and recycling sites.

#### **4.13.P-Mo429 The European Research Cluster to Understand the Health Impacts of Micro- and Nanoplastics - CUSP**

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Micro- and nanoplastics (MNPs) are in the environment and part of our everyday life. They find their way into our body through the food we eat, the water we drink and the air we breathe, yet we currently do not know how they might be affecting human health. They may emanate from the degradation of larger plastic items, or are intentionally manufactured and added to commercial products such as cosmetics, synthetic textiles or paints. Pollutants, such as heavy metals, allergens, toxicants, and microorganisms, can latch on to them and may further endanger the environment as well as human and animal health.

CUSP brings a critical mass of researchers together that are funded through 5 Horizon 2020 projects to investigate the effects of MNPs on human health. While each project has a unique focus all are collaborating closely across a number of areas to maximise impacts. This has included sharing materials, standard operating procedures and results, and participating in interlaboratory studies. Highlights from our work are presented below.

- Developed a list of common materials and SOPs that are available to partners across the projects to access, thus increasing the opportunity to extend and enhance experiments through additional efforts and read across to different systems.
- Contributed to a successful interlaboratory comparison study under VAMAS to assess the ability of different microscopy and spectroscopy methods to accurately measure particle numbers and mass fractions of different plastics.
- Compiled an AI-assisted systematic review of the current evidence on human internal exposure to MNPs in different sample matrices, and identified key aspects of exposure assessment, notably uncertainties in the sample analysis, methodological improvement opportunities, assessing the quality of biomonitoring data and their regulatory relevance.
- Investigated the optimisation of human biomonitoring studies through sharing data and samples and extending the analysis performed by individual partners to benefit the data requirements of others

All CUSP data and metadata are being stored in eNanoMapper to ensure that knowledge gained is accessible for future research. Furthermore, we have published policy briefs that assesses relevant EU regulations and strategies across diverse fields including plastics, chemicals, water, food and the circular economy and identify where CUSP research can help address science-policy gaps.

#### **4.13.P-Mo430 Interlaboratory comparisons for obtaining reliable data on microplastic detection methods**

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Since microplastics can be found everywhere and are becoming a problem of high concern, it is necessary to understand their fate and occurrence in the environment. To obtain reliable analytical data a set of validated methods for sampling, sample preparation, detection, and data evaluation are needed.

One way to address these issues is an interlaboratory comparison. The interlaboratory comparison on microplastic detection methods has been organized under the international pre-standardisation platform VAMAS as Project 2 “Development of standardized methodologies for characterisation of microplastics with microscopy and spectroscopy methods” under the Technical Working Area TWA 45 “Micro and Nano Plastics in the Environment”. In this interlaboratory comparison thermo-analytical methods (Pyrolysis Gas Chromatography Mass Spectrometry and Thermal Extraction - Desorption Gas Chromatography Mass Spectrometry) and vibrational methods ( $\mu$ -Raman and  $\mu$ -Infrared Spectroscopy) have been tested and compared by providing a set of microplastic reference materials and standard operating protocols developed at Bundesanstalt für Materialforschung und -prüfung. It was asked for particle number concentration, particle size distribution, polymer identity and mass fraction. To increase the statistical quality, 6 samples were shipped next to blank samples. The interlaboratory comparison will provide information on precision and accuracy of the methods as well as strengths and weak points in the proposed standard operating protocol.

#### **4.13.P-Mo431 Nanoplastics Affect the Behavior of the Different Cell Populations in Peripheral Blood from Healthy Donors**

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Humans can internalize nanoplastics (NPLs) through inhalation and ingestion due to their small size, allowing for their absorption, reaching the bloodstream, and permitting their systemic biodistribution. Indeed, NPLs have been detected in human whole blood samples representative of the general population.

While some knowledge of the hemocompatibility and inflammatory effects of specific NPLs exists (mainly from *in vitro* studies), humans will be exposed to a heterogeneous mixture of NPLs that varies, among others, regarding the polymer type. Since information is missing, our study aims to characterize the effects of different NPL polymers in human whole blood.

We used an *ex vivo* model in which we exposed human peripheral blood from healthy donors not only to the NPL most used polystyrene but also to polyethylene terephthalate and polylactic acid. To explore the influence of the NPLs physicochemical characteristics on the analyzed effects, different sizes, and surface modified NPLs were chosen. After 24h of blood exposure to NPLs, white blood cells (WBC) were isolated and processed for scRNA seq. Furthermore, as cell-based endpoints, we determined the platelet activation due to NPLs exposure and the internalization rates for each major type of WBC separately as well as the induction of intracellular reactive oxygen species after NPLs *ex vivo* exposure. In addition, we assayed NPLs haemotoxicity measuring the hemolysis and changes in clinical coagulation parameters. Finally, to study potential secretome alterations, we evaluated the expression levels of a panel of representative cytokines.

This study provides the first report of the toxic effects of NPLs on human blood. Through an *ex vivo* model, we have been able to show the different rates of NPLs depending on their polymer nature in the three major WBC (lymphocytes, polymorphonucleated cells, and monocytes). The NPLs toxicity might be associated with ROS and hemolysis generation. In addition, we also report enhanced inflammatory signals by the induction of pro-inflammatory cytokines. Remarkably, the analysis of the scRNA seq data has permitted us to identify differentially expressed genes for the different NPLs exposures, suggesting the influence of NPLs in the production of an inflammatory environment in the body, thus altering human health.

Overall, these findings show us for the first time the NPLs effects on human blood cells and emphasize that may be fundamental to control NPLs exposure.

#### **4.13.P-Mo432 Describing the Fate of Food Packaging-Derived Nanoplastics in the Intestinal Epithelium**

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The increasing presence of micro- and nano-sized plastics (MNPLs) in the environment and food chain, has already been proved and is of emergent concern. Plastics from consumer food packaging can leachate in the form of MNPLs, migrate into the food, and directly enter the human body by the ingestion route. A well-known example is teabags, which has become the main source of MNPLs since the traditional paper bags were substituted with “biodegradable” plastic bags.

However, we have detected an important gap of knowledge on the risk assessment of these true-to-life MNPLs, since the lack of reference materials together with the limited analytical methods available for MNPLs detection has complicated its research. To cover this gap, we have aimed to isolate MNPLs from the leaching of food packaging (teabags) and used them to expose the *in vitro* model of the intestinal epithelium (Caco-2/HT29) to evaluate the fate and effects.

Briefly, four different teabags purchased online and in a local supermarket were submitted to a cup-of-tea-like extraction protocol and then collected in big quantities to perform toxicological assays. The results from SEM-EDX and FTIR confirmed that the particles derived from teabags were made of Nylon, Polypropylene (PP), polylactic acid (PLA), and Cellulose (CS). Moreover, using the Nano Z-sizer we detected hydrodynamic sizes ranging from 120 nm to 250 nm. Finally, TEM and SEM images showed different MNPLs morphologies including spherical PLA-NPLs, amorphous and irregular Nylon- and PP-NPLs and filamentous CS-NPLs. Following, we aimed to study the fate, interaction and biopersistence of those NPLs on human intestine-derived cells. Using confocal microscopy, we confirmed that NPLs could internalize into all the cells after 24 h, reaching the cell nucleus compartment. Flow cytometry data demonstrated that the internalization rate depends on the polymer-type and the cell clone used. For example, while PLA-NPLs internalized 100% of HT29 cell clone, Nylon-NPLs only internalized 60%. Despite the high internalization rate, no significant cytotoxic effects were observed when analyzing cell viability, intracellular ROS, and barrier integrity.

This study opens new insights regarding the fate and effects of MNPLs, reinforces the urgent need for multidisciplinary studies and physiologically relevant exposures, and serves as an example for regulatory agencies to pay close attention to the regulation of MNPLs production and management.

#### **4.13.P-Mo433 Assessment of Pyrolysis-GC-MS as a technique for quantifying *in vitro* uptake of micro- and nanoplastics in human cells**

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Although no direct evidence for adverse effects of micro- and nanoplastic particles (MNPs) on human health has been demonstrated so far, human exposure to MNPs present in the environment via ingestion and inhalation has been shown, and therefore the hazards of these particles to human health needs to be evaluated. To assess potential cellular toxicity of MNPs, *in vitro* testing is commonly performed with human-derived cell lines, however the actual amount of test particles which reach and are taken up into the cells must be determined. Particularly for nanoscale particles (< 1000 nm), handling, particle agglomeration, dosage determination, and most importantly the reliable detection and quantification of actual particle uptake in cells remains challenging. Among other physicochemical properties, particle size is a key parameter influencing cell uptake, and therefore particles in both the micro- and nano-size ranges need to be investigated.

Current detection methods for measuring plastic particle uptake in cells have several limitations, such as the requirement for a fluorescent label for confocal microscopy, a size limitation in the lower  $\mu\text{m}$  range for  $\mu$ -Raman spectroscopy, or challenging sample preparation and limitations for quantification for ToF-SIMS analysis. A commonly available method which enables the detection and quantification of MNP uptake in cells, that overcomes these limitations, is still missing.

Online-coupled pyrolysis gas chromatography mass spectrometry (Pyr-GC-MS) has been shown to reliably quantify MNPs and dissolved polymers in different complex matrices including human blood and *Daphnia magna* phylopopods. Yet, no attempt to use this method for detection and quantification of MNP uptake in human cell culture has been reported. Pyr-GC-MS features polymer quantification over a wide concentration range reaching from ng to  $\mu\text{g}$  quantities, and is also applicable to environmentally relevant MNPs, where the use of e.g. fluorescent labels could lead to changes in surface chemistry and charge and is therefore not appropriate.

We present the development of a Pyr-GC-MS-based method for the quantification of the *in vitro* uptake of polyethylene MNPs of nano- and micrometre sizes in human-derived A549 lung cells. The development of this method focuses on the assessment of linearity, repeatability, and limits of detection and quantification, in order to establish the applicability of Pyr-GC-MS for quantification of MNPs in cell culture toxicity testing.

#### **4.13.P-Mo434 3D In Vitro Tissue Model of Human Alveoli to Assess the Toxicity of MNPLs Following Exposure to the Air-Liquid Interface (ALI)**

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*In vitro* modeling of cell cultures is widely used in pharmaceutical, medical, food/nutrition and toxicology science. These

constructed growth environments support tissue differentiation and mimic tissue-tissue, tissue-liquid, and tissue-air interfaces in a variety of conditions. In toxicology, human-derived *in vitro* culture models are attracting increasing interest because of the numerous benefits including non-animal studies, rapid and cost-effective methodologies, and the simulation of human physiology in a controlled situation. Therefore, our research group has focused on the modeling of *in vitro* barrier tissue interfaces for their use in environmental toxicology. Concretely, we aimed at characterizing and validating a 3D *in vitro* model mimicking the human alveoli. To such end, Calu-3 cells (human lung adenocarcinoma) were seeded on top of a porous (0.4  $\mu\text{m}$ ) transwell and let proliferate and differentiating for 9 days. On day 9, the apical medium was mostly removed recreating an air-liquid interface (ALI). Then, cells were left in ALI conditions for 7 more days, where the barrier was established as a pseudostratified epithelium expressing tight junctions, microvilli and mucosa. Afterward, the 3D *in vitro* model was used to study the effects of MNPLs such as polystyrene (PS), polylactic acid (PLA) and polyethylene terephthalate (PET).

Our results indicate that either after short (24 h) and long-term (1 week) exposures PLA-NPs internalized the most compared to PS and PET. While the presence of PLA-NPLs did not disrupt the barrier morphology nor the stability, significant genotoxic damage was observed already after 24 hours. Most likely, the high internalization rate of these NPLs increased the chances of nucleus-NPLs interactions. PLA-NPLs also induced big cytokine alterations especially after long-term exposures.

Therefore, (i) the ALI model is a useful tool for high throughput screenings and to perform longer and repeated MNPLs exposures; (ii) the presence of mucus might be modulating the differences on adhesion, interaction and cell internalization depending on the polymer-type and its physicochemical characteristics; (iii) PLA-NPLs were detected to induce genotoxicity; and (iv) also dysregulated the cytokine release, which can potentially alters the immune response of the lungs.

#### **4.13.P-Mo435 Characterization of sunlight- and laboratory-weathered MNPs for Toxicological Studies: limits and perspectives**

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The toxicological assessment of micro- and nanoplastics (MNPs) requires test materials that are well characterised and relevant to those found in the environment. Natural weathering of MNPs resulting from different environmental conditions consists of moderate but continued exposure to various environmental stressors such as UV radiation, heat and mechanical abrasion, which may continue for months or years. Importantly, by modulating size, charge and surface chemistry, these alterations can have substantial effects on the uptake of MNPs in cells. To overcome limitations associated with time-consuming and variable weathering under natural conditions, a laboratory-scale apparatus was set up with the aim of producing weathered MNPs with comparable physicochemical properties to those obtained from environmental weathering. The resulting weathered MNPs could then be applied in toxicity assessments. To fill in gaps of information between the outcomes of artificial and natural weathering processes, we investigated the influence of light and media (e.g., deionized water and artificial seawater) on microplastics weathered either artificially in the laboratory or naturally via exposure to summer sun. In particular 1–4  $\mu\text{m}$  polyethylene (PE) particles in artificial seawater or in MilliQ water were exposed for 770 h to UVA/UVB light (115  $\text{W}/\text{m}^2$ ,  $T = 63 \pm 5$  °C, stirred at 460 rpm), and, as powder, to natural summer sunlight in Portici, Naples (40°48'56" N; 14°20'13" E,  $T = 29.8$  °C, UV radiation index = 5.1) for 12 weeks without stirring. A variety of analytical methods (including FTIR, SEM and ToF-SIMS analysis) were used to compare the physicochemical properties of the aged MNP variants as well as non-degraded particles. The MilliQ and artificial seawater media in which particles were dispersed during the exposure period were also investigated (by GC MS and ICP MS analysis) to determine the presence of leached organic and inorganic species. To improve our understanding about how particles resulting from different types of weathering can influence MNP-induced cellular effects, we also investigated ROS production, lysosomal membrane stability and cell viability in MNP-exposed *Mytilus galloprovincialis* hemocytes.

#### **4.13.P-Mo436 Micro-nanoplastics, their effect on composition and metabolic functionality of human microbiota and microbial-derived metabolites' impact on the gut barrier**

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In recent years, the presence of micro- and nanoplastics (MNPs) has been detected almost ubiquitously. As environmental contaminants, these particles can be deposited on food along the production and consumption chain. Concomitant unconscious ingestion could have yet to be elucidated effects on human health. In addition, pathogens capable of surviving hostile conditions may colonize MNPs, establishing a microbial biofilm and using it as a route of transmission. The interaction of either pristine or colonized particles with gastrointestinal fluids and/or gut microbiota during human digestion is still unknown.

In this intergroup collaboration, relation between MNPs ingestion, their physical changes during digestion and by colonic microbiota and its microbial-derived metabolites was examined *in vitro*. Firstly, PET particles were subjected to gastrointestinal digestion using INFOGEST method with modifications for MNPs. Later, colonic fermentations with healthy human microbiota from fresh fecal donors was carried out. Furthermore, the digested particles were exposed to the Caco-2/HT29-MTX/M intestinal barrier cell model to evaluate their effects on cell viability and barrier permeability and integrity. On the other hand, colonic effluents supernatants were tested in the same way to assess the effect of microbial metabolites as result of PET MNP exposure.

Finally, the simulation of *in vitro* colonic fermentation with artificial pathogen biofilms was carried out. Further analyses will evaluate their impact on human health by studying the composition and functionality of the colonic microbiota (16S gene meta-taxonomy), short chain fatty acids and ammonium ion production, as well as by exposure of colonic supernatants in intestinal cell models.

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#### **4.13.P-Mo437 Three-Dimensional A549 Cell Cultures to Study the Chronic Toxicity of Micro- and Nanoparticles in the Respiratory System**

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Air pollution is the main cause of the development and exacerbation of respiratory diseases such as asthma, bronchitis, and chronic obstructive pulmonary disease. Recently, scientists have detected the presence of microplastics (MPs) and nanoplastics (NPs) in various outdoor and indoor air settings. Consequently, there is a growing concern over the access of these particles into the respiratory system through inhalation.

In this study, a 3D human alveolar epithelium adenocarcinoma A549 model was established to evaluate the chronic toxicity of polystyrene MPs (1  $\mu\text{m}$ ) and NPs (50  $\mu\text{m}$ ), with a special focus on changes in cell lipidome and the transcription of genes involved in cell proliferation pathways and pro-inflammatory cytokines. Spheroid growth was monitored for 14 days. Spheroids aged 6 days, with a size of approximately 400  $\mu\text{m}$ , were chosen for toxicity tests, which by the end of the experiment (13 days), increased to  $642 \pm 20 \mu\text{m}$ . Chronic exposure (7 days) of MNPs (2.6  $\mu\text{g}/\text{mL}$ ) did not significantly affect spheroid size nor decrease cell viability. Nonetheless, exposure to polystyrene NPs significantly upregulated various lipid species, particularly triglycerides (TGs) (up to 1.5-fold) and plasmenyl/plasmanyl phosphatidylcholines (PC-O/PC-Ps) (up to 1.5-fold). The accumulation of neutral lipids (TGs) is linked to lipid storage and the development of lipid droplets within lung cells. Meanwhile, PC-P/PC-Os are crucial in neutralizing free radicals and safeguarding against oxidative damage, and they are also precursors of pro-inflammatory factors. Overall, the study highlights the potential of the A549 spheroid model for assessing the effects of MPs and NPs on lung toxicity and provides insights into lipidomic and molecular alterations in response to these particles.

#### **4.13.P-Mo438 Polyethylene Terephthalate Microplastics Alter Adrenal Transcriptome Profile Of Immature Gilts – In Vivo Studies**

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Polyethylene terephthalate (PET) is the most commonly used polymer in the packaging industry. PET microplastic particles (MPs) can be released during food storage and few studies confirmed their accumulation in human tissues and blood. Polymers have been shown to disturb physiological processes in living organisms, therefore the aim of our study was to investigate whether oral administration of PET MPs affects the transcriptome profile of the adrenal glands of immature gilts.

The experiment lasted 4 weeks and was conducted on 8-week-old (Pietrain x Duroc) immature gilts (n = 15) with an estimated body weight of 20 kg. The animals were divided into three groups: 1) control group (CTR; n = 5), which received empty gelatin capsules per os; 2) experimental group (LD\_PET; n = 5), which received a low dose of PET MPs (0.1 g/pig/day) per os; 3) experimental group (HD\_PET; n = 5), which received a high dose of PET MPs (1 g/pig/day) per os. The plastic used for the experiment is a semi-crystalline polyethylene terephthalate powder (Goodfellow Cambridge Ltd., Huntingdon, England). To determine the expression profiles of the adrenal genes, RNA-Seq was performed on the NovaSeq 6000 Illumina platform. To identify differentially expressed genes (DEGs), 74 Cufflinks method was used. The final results represented DEGs that were significantly confirmed by statistical test (p-value < 0.05; log<sub>2</sub> fold-change > 1,5 or log<sub>2</sub> fold-change < -1,5). Gene ontology (GO) enrichment and pathway analysis were performed using gProfileR based on the GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.



The statistical analysis revealed 344 DEGs (101 up and 243 down) in adrenals of LD\_PET group relative to control group. Selected genes were related to regulation of anoikis, CREB transcription factor activity or cellular response to glucose starvation. We identified 628 DEGs (161 up and 467 down) in adrenals of gilts of HD\_PET group relative to control individuals. The GO analysis revealed that these genes refer to the biological processes connected with chemokine-mediated signaling pathway, anatomical structure development or antimicrobial humoral response. These findings, for the first time, indicate that PET MPs can interrupt basic physiological processes in the adrenal gland of immature organism, and in consequence may cause serious endocrine disorders.

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#### **4.13.P-Mo439 Development and Understanding of Adopted Bioassays for the Hazard Assessment of Plastics in the Environment**

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Plastics, especially particles in micro- and nanometer range, are persistent contaminants in ecosystems, with only incomplete information available particularly on their specific hazards. In most cases plastics are equipped with additives and further chemicals. Plastic containing waste is a broad combination of polymers, additives and further compounds. Therefore, eluates from shredded plastic waste can serve as a model substance to develop and optimise standardized bioassays for assessing hazards of plastics.

Plastic containing waste of two different waste treatment plants were fractionated (</>300µm) and aqueous extracts were prepared. The study used bioassays (e.g. algae growth inhibition test, in vitro test for estrogenicity) and chemical analyses to profile organic pollutants, heavy metals and other chemical constituents of extracts.

Algae growth rate inhibition showed higher effects for extracts of the smaller fraction, with no differences between the two plants. Extracts of unfractionated samples caused comparable growth inhibition as the smaller fraction, indicating a major part of ecotoxicologically relevant compounds are present in this fraction. Assuming particles of this fraction have a higher surface to volume ratio, stronger ecotoxicological effects should be caused by surface-controlled release of pollutants into the extract. In turn, estrogenicity results revealed strong differences between both tested plants, but remarkably nearly no difference between different fractions. The latter indicates the surface-controlled release of pollutants in the eluate not to be the underlying effect.

Chemical analyses confirmed higher concentrations of anionic substances and several heavy metals (e.g. copper) in the smaller fraction of one plant. For the same plant relatively high contents of estrogenic substances, particularly bisphenols, were detected. As no difference between the different fractions was observed, the migration kinetics of bisphenols, as additives of polymers, are suggested to be responsible for their concentrations in the eluate.

In sum, established bioassays can be used for the hazard assessment of plastics (here shredded plastic waste as model substance) in the environment. However, a suitable method for converting potential pollutants into an eluate is essential. Further, fractionation of samples can alter ecotoxicological and chemical results probably due to particle size and compound migration dependent extraction mechanisms.

#### **4.13.P-Mo440 Immunomodulatory effects of micro-and nanoplastic particles (MNPs) in human immune cells**

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Background: Micro- and nanoplastic particles (MNPs) have been frequently shown to be taken up by various human cell types, thereby affecting inflammatory responses such as distinct levels of reactive oxygen species (ROS), which might be protective and/or toxic. This raises the question of possible harmful molecular and cellular effects of MNPs on human immune cells and their (patho-)physiological signalling pathways.

Methods: To address this issue within the PolyRisk Project, molecular and cellular in vitro studies were carried out with human immune cells. In a two-tiered approach we investigated molecular effects of polystyrene MNPs (PS-MNPs, 100 nm size) on selected enzymes e. g. of cellular apoptosis, and on monocytic cells. Human immune cells were derived from peripheral blood mononuclear cells (PBMC), and analysed by flow cytometry (FACS, fluorescent activated cell sorting).

Results: (1) Biochemical studies showed an inhibitory effect of PS-MNPs on a key enzyme (caspase) of cellular apoptosis. (2) Flow cytometric studies after exposure of PBMC to PS100nm showed e.g. an increased expression of the surface molecule CD40, which is obligatory for antibody class switching during human type I allergic reactions. In addition, a clear shift in the distribution of human monocyte subsets was observed after PS-MNPs stimulation. All experiments were performed at least with n=3; and data were compared with standard LPS (lipopolysaccharide) stimulation.

Conclusions: The results to date suggest that (1) PS-MNPs interfere with enzymes responsible for apoptotic cell death; (2) an immune cell response to PS-MNPs occurs, as indicated by the upregulation of CD molecules, e.g. CD40; (3) an

immunomodulatory response to monocytic subsets from human PBMC occurs, which has been also observed under septic conditions.

Since plastic polymers are usually chemically inert, there is probably an indirect mode of action for triggering the effects described above and previously. A closer look at different activation markers and phenotype distributions could (1) unravel the underlying mechanisms and (2) serve as a potential descriptor for future risk assessment frameworks related to MNPs and certain cellular human immune responses.

Without chemical activity and without structures suitable as receptor ligands, PS-MNPs (100nm) may have triggered the response by affecting endosomal homeostasis upon uptake, due to possible enzyme adsorption. This will be subject of further investigations.

#### **4.13.P-Mo441 Identification of toxicologically relevant functional groups on micro- and nanoplastic particles surface**

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Microplastic and nanoplastic particles (MNP) are spread all over the world in various types, shapes and sizes making it very challenging to accurately analyse them. Each sampling procedure, sample preparation method and detection technique needs suitable reference materials to validate the method for accurate results. Furthermore, the effects of these MNPs should be evaluated by risk and hazard assessment with test particles close to reality. To better understand MNP behavior and contribute in clarification of their interactions with organisms, we produced several MNP test materials by top-down procedure and characterized their properties. Since surface properties mostly determine particles' toxicity, the aim of the present study was to determine which functional groups are present on MNPs and how the surface can be affected by the production process and particle's environment.

Our study showed that the production process influences the surface properties of every plastic particle. Furthermore, analysis of surface characteristics of the tested particles seems to be mandatory for interpretation of experimental results. Especially toxicological experiments should be supported with information on surface properties.

#### **4.13.P-Mo442 PolyAmidst the crisis: Exploring immunotoxic, genotoxic, and endocrine disrupting effects of polyamide microplastic particles and chemicals**

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Due to their outstanding characteristics and cost-effectiveness, polyamides or nylons have become prevalent materials, transforming various industries, including industrial 3D printing, aka additive manufacturing (AM). Powder-based AM technologies utilize tons of polyamide microplastics annually to manufacture complex components. However, the urgent need for a comprehensive toxicity assessment of particulate polyamides and associated chemicals, particularly amidst the global microplastics crisis, is evident. Hence, this research delved into the physicochemical properties of polyamide-12 microplastic particles employed in AM by focusing on toxicity endpoints, such as inflammation, immunometabolism, genotoxicity, aryl hydrocarbon receptor (AhR) activation, endocrine disruption, and cell morphology. Field emission scanning electron microscopy revealed that the reuse of material in the AM workflow generate smaller particles (1-5 µm), readily available for the uptake by human cells. Chemical analysis via gas chromatography high-resolution mass spectrometry identified various polyamide-associated chemicals, including starting material, plasticizers, thermal stabilizer/antioxidant, and migrating slip additive. Although polyamide particles and chemicals did not induce acute inflammation, prolonged exposure of human primary macrophages led to a steady increase in proinflammatory chemokine interleukin-8 (IL-8/CXCL-8). Additionally, targeted metabolomics indicated modulation of the kynurenine, NAD, and FAD levels. A p53-responsive luciferase reporter gene assay revealed that prolonged exposure to polyamide particles elicited genotoxic stress. Polyamide-associated chemicals moderately activated AhR and exhibited anti-androgenic activity. A high-throughput, non-targeted morphological profiling by Cell Painting assay highlighted bioactivity sites and suggested potential toxicity mechanisms in cells. These findings underscore the potential health risks associated with the growing use of polyamide microplastics.

#### **4.13.P-Mo443 Developmental Nanoplastic Exposure Disrupts the Thyroid Hormone Axis in Zebrafish Embryos**

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Worldwide plastic production has greatly increased in the past decades. Mechanical, physical, and biological processes degrade this material producing Micro- (MP) and Nanoplastics (NP). The ubiquitous nature of MP and NP is a growing environmental and human health concern. The small size of NPs allows them to accumulate within and adversely affect

tissues, causing disruptions in metabolism, development, and fertility. Mechanisms of NP toxicity are largely unknown and the zebrafish embryo (ZFE) poses an ideal model to investigate them due to its high homology with humans.

The present work was aimed at determining the potential impact of developmental exposure to polystyrene (PS) NP on the thyroid endocrine system in ZFE. To this end, embryos were exposed to a concentration gradient of PS NP (0 -3 mg/L) up to 120 hours post-fertilization. After exposure, alterations in gene transcriptions and thyroid hormones (THs) were examined. Our results show that exposure to PS NPs decreased the levels of thyroid stimulating hormone (TSH) and L-triiodothyronine (T3); whereas L-thyroxine (T4) varied depending on NP concentration. In addition, the T3/T4 ratio was affected. Furthermore, we observed that expression of several transcripts related to the thyroid axis were modified. Together, these results provide further evidence of a potential mechanism of action of NPs as endocrine disruptors and highlight the pressing need for more research on potential human health effects.

#### **4.13.P-Mo444 Short-term exposure to polystyrene micro- and nanoplastic (PS MNP) – in vivo uptake and effects in a colitis mouse model**

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Micro- and nanoplastic particles (MNP) have been found in recent years in all parts of the world and in various organisms. In the human body MNP have been detected e.g. in stool, blood, placenta, kidney or lungs - not surprising considering that we consume on average up to 5 grams (!) of MNP per week. Until now, the health effects of this exposure to MNP are almost completely unexplored, especially regarding tumor development, growth and dissemination. For the gastrointestinal tract, as one of the organs that is exposed to high amounts of MNP on a daily basis, already MNP related changes to the gut microbiome, barrier function and inflammatory processes have been described – all three being factors potentially influencing (colorectal) tumor development and progression.

Thus, our main research interest within this project is to explore the potential health risks of various MNP types, with a special focus on colorectal cancer development and progression, using in vivo mouse models. First short-term exposure studies were conducted with polystyrene (PS) MNPs of three distinct sizes. Uptake and potential accumulation in various organs was assessed in mice using a DSS-induced colitis model, which mimics increased MNP uptake due to an inflamed/leaky gut barrier. As a future perspective also colorectal mouse models (genetic an inflammation based) will be used to study the relationship between MNP and colorectal cancer in more detail.

#### **4.13.P-Mo445 Development of Analytical Methods for the Quantification of Microplastics in Animal Feed Containing Former Food Products**

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Considering the current increase in population accompanied by high food demand/production and food waste, the long term goal of our society is to encourage the practice of an increasingly Circular Economy. Recycling food no longer suitable for sale and human consumption due to logistical or production errors (for example packaging defects), are indicated as former food products (FFPs). These foodstuffs are recovered, mechanically unpacked, and then ground. This treatment, however, does not always guarantee the total elimination of packaging residues that can become part of the product and potential contaminants. Plastic is the most used food packaging material but its carry over into food products and subsequent transfer in the food chain, especially in its micro sizes (1  $\mu\text{m}$  – 5 mm), represents a threat to human health. To maximise the exploitation of former food products and minimise the associated toxicity risks, the development of analytical methods for the identification and quantification of microplastics in animal feed containing FFPs, for which limited data are available so far, is necessary. Twelve animal feed samples were analysed, including blank sample. After a manual sift through different mesh sizes (2, 1.3, 1, 0.7 mm) and visual sorting procedure, presumed plastic packaging residues in the form of fragments and fibres (main forms of plastic in food) were analysed by  $\mu\text{Raman}$  (DXR-xi Raman Imaging Microscope, by Thermo Scientific). The analysis confirmed the presence of plastic residues identifying polypropylene (PP), polyethylene terephthalate (PET), polylactate acid (PLA) as the main polymers, with a predominance of polyethylene (PE). In accordance with the European Commission Agency's size classification of plastics, an abundance of microplastics (1  $\mu\text{m}$  – 5 mm) and remaining low amount of mesoplastics (> 5 mm) were found in the analysed samples.

The results showed that  $\mu\text{Raman}$  measurements provide quantitative and qualitative analyses of plastic packaging residues. Accordingly, it can be considered as an innovative method to investigate their presence in feed and food by supporting the protection of animal, plant and environmental health, which represents the basis of public health. The future prospective this study also aims at the development of method of digestion of the same samples in their sieved component < 0.7 mm, to investigate the presence of submicron sizes (1  $\mu\text{m}$  – 0.7 mm) packaging residues.

#### **4.13.P-Mo446 Asymmetric Field Flow Fractionation-Dielectrophoresis-Raman Combination for Chemical Identification of Nanoplastics in Aqueous Matrices**

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Nowadays, identification, quantification and characterization of micro (MPs) and nano-plastics (NPs) in food and environmental matrices have been gaining a lot of attention due to their suspected toxicological effect on human health. While several studies have been conducted on MPs, for NPs big challenges have to be taken in consideration because of their diversity in size (1 nm – 1 µm), shape, and type of polymers. In this work, we demonstrate the feasibility of using Raman microspectroscopy (µ-Raman) enabled by dielectrophoresis (DEP), which allows the trapping of particles, to chemically identify NPs up to 100 nm in diameter. Furthermore, binary mixtures of NPs, in particular nano-polypropylene (nPP) and nano-polystyrene (nPS), have been developed, proposing different percentages of them. These nanoparticles were selected in the same size range, approximately 200 nm, as demonstrated by dynamic light scattering measurements. A multivariate predictive model will be trained and validated using these mixtures. Then, size separation of NPs was obtained by asymmetric flow field-flow fractionation (AF4) and the collected fractions were analyzed by multi-angle dynamic light scattering to have information about their size and size distribution. The chemical composition of the same collected fractions were analyzed by µRaman enabled by DEP, as explained above. The multivariate predictive model will give information about relative concentration of nPP and nPS in collected fractions. At the end of this study, we will have the possibility to chemically identify both NPs smaller than 200 nm up to 100 nm, as well as NPs with the same size but different polymer type in the same mixture, obtaining their relative quantification.

#### **4.13.P-Mo447 Standardization Methods for the Analysis of Microplastics (10-100µm) in Food Matrix: Sample Preparation, Contamination Control and Digestion of Milk Powder.**

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The ubiquitous presence of microplastics in the environment is recognised as a global issue. Microplastics have been found in each environmental compartment and also in humans. The direct exposure of microplastics to the people due to the food matrix has gained more and more attention in the last years. Although the presence of microplastic in various human tissues has been found, the information on the ingestion of microplastics due to the food matrix has been scarcely investigated. Microplastics in fact could be released in the food during the production, through packaging and by consumer's use. The lack of data and their comparison, is mainly due to the methodological challenge of microplastics' properties (from 10 to 100 µm). Moreover, the absence of standard methods to quantify and detect different size range and type of microplastics has led to series of analytical issues such as difficult and time-consuming procedural steps and poor accuracy. In this work, through literature research and laboratory experiments, we compile a standard procedure, for the contamination control, sample preparation and digestion of common milk powder to detect different particle sizes and types of polymers. This information is crucial for establishing a standard method that would determinate the human exposure to microplastics, by direct ingestion, from the earliest years of life.

#### **4.13.P-Mo448 Proposal of exposure reduction measures according to microplastic exposure scenarios in household chemical products**

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Recently, as microplastics have been recognized as a serious environmental problem, interest in microplastics is increasing around the world. Currently, research on microplastic exposure assessment in living environments is insufficient, and it is difficult to secure microplastic exposure assessment methodology and applicable exposure coefficients. Accordingly, in this study, a data research study was conducted to obtain information that can be used to evaluate exposure to microplastics during living environment activities, including exposure coefficients, exposure routes, and exposure scenarios. Products that have the potential to cause microplastic exposure to the human body and the environment, such as household chemicals, laundry detergents, detergents, microfibers, disposable masks, and sanitary products, were selected as the target products for this study. A survey was conducted on the selected products to derive exposure factors. The purpose of the survey was to set the exposure scenario, derive the exposure factor, and obtain the necessary information to set the exposure assessment model, and the survey was conducted by dividing the questionnaire according to the characteristics of each target product. Additionally, we sought to identify possible factors that could reduce exposure according to the exposure scenario derived from the survey results. We propose a management plan that reflects the use scenarios for products containing microplastics identified through this study and international regulatory trends on microplastics.

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Keywords: Microplastics, Household chemical products, Exposure scenario, Exposure reduction measures

#### **4.13.P-Mo449 Granulated rubber used in playgrounds: a potential source of atmospheric contamination**

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Granulated "crumb" rubber sourced from recycled tyres and other elastomeric materials such as ethylene propylene diene

monomer is versatile and its many uses include installation as cushioning surfaces and synthetic turf infill in environmentally sensitive areas such as children playgrounds and sports facilities. When installed outdoors, these artificial surfaces are subjected to physical and chemical degradation by weathering during which chemical pollutants can be released.

In this context, we exposed seven granulated and 3 resin-agglomerated crumb rubber materials to natural weathering effects between June and July 2021 in Barcelona city (NE of Spain). The samples were taken using three hung Personal Monitoring Equipments air samplers equipped with quartz fiber filters of PM<sub>2.5</sub> (n=2) for analyses of micro- and nanoplastics (MNPLs) and plastic additives and PM<sub>10</sub> (n=1) for inorganic analysis. In addition, the toxicity of aged material after emulating rain events was assessed with *Daphnia magna* immobilization test.

The studies of MNPLs and plastic additives were based on suspect screening procedures. PM<sub>2.5</sub> filters were extracted by toluene and analysed by size exclusion chromatography coupled to high resolution mass spectrometry (HRMS) for MNPLs while for plastic additives these were extracted with methanol for further analysis by liquid chromatography coupled to HRMS. Finally, the analysis of trace elements was done by ICP-AES and ICP-MS.

The main results showed the presence of 5 polymer types that were lixiviated from crumb rubber including PE, PP, PBD, polysiloxanes and PB-1, varying the profile along the exposition days, denoting that MNPLs can be detected in the air due to the natural erosion of crumb rubber. In the case of plastic additives, the presence at level 2 of confidence of 56 plastic additives including adhesives, antioxidants, pigments, copolymers, flame retardants, fungicides, lubricants, plasticizers, polymer preservatives and UV filters were identified. As regards to the elemental composition of PM<sub>10</sub>, the levels were notably higher on the first day of the experiment compared to the rest of experiment.

Finally, the immobilization tests showed that the acute toxicity with *D. magna* of leached compounds after raining period, either virgin material or aged material, are toxic for the organisms. However, the concentrations tested here were much higher than the ones expected to occur during rainstorm events in a playground.

#### **4.13.P-Mo450 Exposure to Tire Rubber Compounds via Dermal Contact**

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Due to the wide use of recycled tire crumb rubber (RTCR) as infill in turf fields and inurban recreational pavements, tire rubber compounds are in direct contact with people, especially with children that are the main user of this type of facilities. The European legislation only limits the content of 8 PAHs (Polycyclic Aromatic Hydrocarbons) to 20 µg/g in this type of materials. Besides, for other compounds, such as antiozonants, vulcanizing and crosslinking agents, there is no current regulation. However, in September 2023 the European Commission announced the ban of the use of crumb rubber as infill allowing manufacturers eight years to adapt to the new regulation.

The aim of this work is to assess the exposure to several hazardous chemical such as PAHs via dermal contact. To evaluate this, wipes and cotton pads are used to clean children's hands after playing in artificial turf football fields. Two different techniques, Pressurized Liquid Extraction (PLE) and Ultrasound Assisted Extraction (UAE) are used to extract the wipes and pads. The identification and quantification of the target analytes is performed by gas chromatography coupled to tandem (GC-MS/MS).

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#### **4.13.P-Mo451 Microplastic polyethylene induces hearing and sensorimotor deficit in mice**

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Balance and hearing loss are not uncommon in any of the age groups. The hazardous effects of microplastics (MPs) are increasingly reported; however, whether the balance and hearing loss are induced by MPs remains unknown. In this study, we investigated whether MP polyethylene induced hearing loss in a mouse model. To detect hearing loss and balance defect after polyethylene (PE) exposure, we evaluated genetic changes, hearing levels, and cerebral glucose metabolism using auditory brainstem responses and <sup>18</sup>F- fluorodeoxyglucose (FDG) PET in addition to behavioral study. C57BL/6J mice (5-week-old) were grouped into control (n = 10) and PE-fed groups (n = 10). Mice were orally administered 100 ppm/100 µL of PE every day for 4 months. We identified the accumulation of PE in the cochlea and vestibular region. The fragmented PE in inner ear

was  $3.00 \pm 0.38 \mu\text{m}$  in size; the administered PE concentration was  $1.14 \pm 1.06 \text{ mg/g}$ . Fourier transform infrared spectrometry confirmed that the properties of the recovered MP were similar to those of PE fed to the mice. Exposure to PE increased the hearing thresholds and decreased glucose metabolism in the bilateral lateral entorhinal cortex, right primary auditory cortex, and right secondary auditory cortex. We observed changes in gene expression levels and abnormalities in balance related behavior assessment in the PE group. Therefore, PE exposure induces hearing loss and affects balance. MP environmental pollution could be one of the causes of hearing loss and balance defects.

#### 4.13.PC The Fate and Effects of Micro- And Nano-Plastics in Relation to Human Health Exposure

#### 4.14 Understanding, Detection, Monitoring, and Management of Harmful Algal Blooms (HABs) and Biotoxins for a Safer Environment and Public Health

##### 4.14.T-01 Abundance and Co-variation of Toxins and Secondary Metabolites from Cyanobacteria of 4-year Lake Study

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Cyanobacterial blooms are increasing globally, posing threats to aquatic ecosystems and human health especially by the release of toxins. Besides widely studied microcystins, a legion of other secondary metabolites has been identified over the past decades including anabaenopeptins, cyanopeptolins and microginins among others. However, we still lack data on their co-occurrence in surface waters. Here, we investigated the diversity and the temporal trends of co-produced metabolites across a four-year lake study. We performed target analysis and suspect screening with high-resolution mass spectrometry on lake samples using CyanoMetDB, a comprehensive, open-access structural database of known metabolites from cyanobacteria. Overall, the lake was dominated by 9 target toxins and 27 suspect metabolites. While [D-Asp<sup>3</sup>, E-Dhb<sup>7</sup>] MC-RR was the most abundant microcystin, Oscillamide Y, an anabaenopeptin, occurred at up to 100-fold higher concentrations, which can be further considered as an indicator substance for the monitoring program of this lake. Our results further indicate that anabaenopeptins and microginins were dominant co-occurring metabolites in this lake with oscillating concentration profiles throughout the year but with opposing relative abundances. We use hierarchical clustering and correlation analysis to group metabolites by their temporal trends and further explore relationships with physical-chemical water quality parameters and phytoplankton species composition that we recorded along with the metabolites data. We demonstrate the complexity of toxin/metabolite profiles across seasons and years with new implications for selecting indicator substances in monitoring programs. Such data open new opportunities to explore the link between relative abundances of cyanotoxins/metabolites and the evolution of bloom dynamics also for the development of early-warning strategies.

##### 4.14.T-02 Genetic and Metabolic Diversity of Cyanobacteria and Their Toxins on Rock-water Interfaces in Mountain Habitats

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Subaerial, often dark-colored surfaces, termed *Tintenstrich communities* (TCs), extend on rock faces in semi-aquatic environments. These communities are widely distributed in high mountain areas, including large parts of the Alpine region, and are predominantly composed of free-living cyanobacteria and associated with lichen-forming fungi (e.g., cyano-lichens). Toxins of cyanobacteria are an ongoing subject of studies in surface waters around the world, as they pose a major concern for environmental and public health, and the World Health Organization defined limits for cyanotoxins in drinking and recreational water quality guidelines. Despite that, many questions remain unsettled about their role at the land-water interface, and its occurrence in the Swiss Alps is currently unknown. Therefore, we explore for the first time, the genetic diversity of cyanobacteria and the metabolic diversity of toxins and other bioactive compounds in alpine regions. A total of 214 TCs specimens examined exhibited the presence of cyanobacterial strains (16S rRNA gene sequencing). PCR analyses suggest that 30% of samples were positive for gene encoding for anatoxin (atxA), 29% for cylindrospermopsin (cryJ) and 25% for microcystins and/or nodularins production (HEPF). Methanolic extracts of samples that obtained positive results for genes associated with toxin production were analyzed by online-SPE-LC-MS/MS. Suspect-screening against CyanoMetDB was used to annotate tentative candidates. Cyanotoxins and other bioactive metabolites from cyanobacteria were detected in the majority of the sampled areas. Variants of anatoxins were identified in the metabolic screening in 84% of all analyzed samples obtained from siliceous rock. In contrast, anabaenopeptins were most frequently detected in limestone samples, which suggests that substrate type may select for different species. Our results present the first empirical data on TCs toxin production in alpine regions. Further studies are required to understand the selection pressure of biosynthetically different cyanobacteria in these areas and, our obtained cyanobacterial isolates from these sites will facilitate such investigations.

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#### 4.14.T-03 Zebrafish Larvae Exposed to BMAA and Isomers DAB and AEG: A Morphological and Behavioural Study

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BMAA (beta-methylamino L-alanine) is a neurotoxin produced mainly by cyanobacteria and the subject of several hypotheses suggesting a link with amyotrophic lateral sclerosis (ALS). It has historically been found in cycad roots, consumed in various ways by the Chamorros on the island of Guam, where the incidence of ALS is much higher than the global average. Other clusters have also been identified by the scientific community and several studies have highlighted multiple mechanisms of toxicity of this molecule. Two isomers are also identified and studied: DAB (2,3-duaminobutyric acid) and AEG (N-(2-aminoethyl) glycine).

In France, in the Occitanie region, BMAA and/or isomers have been detected in the Thau lagoon in bivalves and, depending on the time of year, in water and/or air samples. Other global studies show the possible presence of toxins in the environment at nanogram concentrations. As highlighted by the 2017 French National Agency for Food, Environmental and Occupational Health and Safety report, information on the effects of environmental concentrations remains to be completed.

We therefore tested BMAA, DAB and AEG on a common use toxicological model: *Danio rerio*; at the doses of 1-10-100 ng.L<sup>-1</sup> and 1-500 µg.L<sup>-1</sup>. Zebrafish larvae were exposed from 28 to 100 hours post-fertilization. A morphological analysis evidenced effects (e.g. edema, scoliosis, etc.) of each toxin even at the lowest concentrations. Additionally, the swimming behaviour of exposed zebrafish was studied (Daniovision®). Larvae were observed for three minutes of free swimming, followed by a tap stimulus and two more minutes of free swimming. Effects on swimming distance have been highlighted for the three molecules, confirming an impact of the toxins at the nanogram concentrations. Statistical significance differed depending on the parameter analysed (total distance, distance covered 1 or 5 seconds before and after the tap stimulus). From these results, DAB and AEG seem to have a higher toxicity if compared to BMAA as their impact is stronger and highly statistically significant. To note, these data suggest also a nonlinear dose-response relationship, supporting the value of studies at environmental concentrations. To our knowledge, this is the first study to focus on such low doses.

Overall, the morphological and behavioural analysis show complementary results and indicate the importance of pursuing research.

#### 4.14.T-04 Spatiotemporal Distribution and Driving Mechanisms of Algal Toxins and Their Producers in Hong Kong Coastal Waters

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The global incidence and impact of harmful algal blooms (HABs) are escalating, primarily driven by anthropogenic pollution and climate change in coastal regions. Lipophilic algal toxins (LATs) produced by toxigenic microalgae pose threats to marine ecosystems and human health. However, the ecological dynamics influencing HAB trends and patterns at both global and local scales remain poorly understood as the effects of climate change and eutrophication differ among species. Hong Kong, renowned for its diverse marine ecosystems and thriving aquaculture industry, understanding the spatiotemporal distribution and the underlying mechanisms of algal toxins and their producers is crucial. To address this, a comprehensive investigation of LATs in Hong Kong's coastal waters was undertaken, employing a DNA metabarcoding technique to identify potential toxin producers during wet and dry seasons. Among the nine targeted LATs, pectenotoxin-2 (PTX-2), okadaic acid (OA), dinophysistoxin-1 (DTX-1), and gymnodimine (GYM) were detected in surface seawater across two seasons, with concentrations ranging from 0.064 to 8.07 ng.L<sup>-1</sup>. PTX-2 and OA emerged as the predominant toxins, collectively contributing to more than 70% of the total detected LATs. Notably, PTX-2 concentrations were significantly higher in the dry season throughout the entire study area. Significant spatial variations occurred in the concentrations of OA, DTX-1, and GYM, with the eastern waters exhibiting the highest levels. Of the 35 potentially harmful algal taxa identified, 25 showed a significant correlation with the detected toxins. The analysis further highlighted that *Dinophysis spp.* and *Alexandrium spp.*, known potential producers, exhibited a positive correlation with the concentrations of PTX-2 and DTX-1. Similarly, a positive correlation was observed between *Gymnodium spp.* and GYM levels. These related harmful taxa contributed more to the LATs than environmental parameters based on variation partitioning analysis and hierarchical partitioning (42%). Among the environmental factors screened, temperature and salinity emerged as key influencers on the harmful taxa, thereby regulating the distribution of toxins. These findings hold significant relevance for predicting how toxigenic marine algae may respond to future changes in the coastal environment, thereby providing valuable insights for the management and conservation of marine ecosystems in the face of climate change and anthropogenic pollution.

#### 4.14.T-05 Mixtures of Organic Micropollutants Exacerbated the In Vitro Human Neurotoxicity of Pymnesins and Contributed to Aquatic Toxicity During a Massive Fish Kill in the Oder River in 2022

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Prymnesins produced during an the algal bloom by *Prymnesium parvum* led to the death of several hundred million tons of freshwater fish in the Oder River in summer 2022. We investigated effects on aquatic life and human health from exposure to contaminated water or fish. Prymnesins and >120 organic micropollutants were quantified in water samples collected during the peak of the fish kill. Chemicals from diverse sources led to mixture effects with the predicted mixture risk quotient exceeding the threshold of one. Extracts of water and filters (biomass and particulates) induced moderate effects *in vivo* in algae, daphnids and zebrafish embryos but caused remarkably high effects in a human neuronal cell line indicating the presence of neurotoxicants. Prymnesin standards and standards of the detected chemicals were tested with the *in vitro* neurotoxicity assay. Mixture toxicity modeling demonstrated that the neurotoxic effects were mainly caused by the detected prymnesins with minor contributions by the detected organic micropollutants. Extracts from gills and muscle of exposed fish from the Oder River were only moderately neurotoxic and prymnesins could not be detected in fish. The high neurotoxicity of water and extracts of algal biomass is a warning sign that human health could be compromised by exposure to water and fish during hazardous algal blooms. The high toxicity of prymnesins was exacerbated by the high micropollutant loads that clearly led to mixture effects in cytotoxicity and neurotoxicity assays based on differentiated human neuronal cells. However, organic micropollutants were not deemed to be a major contributor to the actual fish kill due to the high ichthyotoxicity of prymnesins that clearly dominated.

#### **4.14.P Understanding, Detection, Monitoring, and Management of Harmful Algal Blooms (HABs) and Biotoxins for a Safer Environment and Public Health**

##### **4.14.P-Th449 Bloom Dynamics Monitoring Program of the Toxic Benthic Dinoflagellate *Ostreopsis* along the Basque Coast (South East Bay of Biscay)**

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*Ostreopsis* is a potentially toxic benthic dinoflagellate genus that, originally, was mostly distributed in warm and tropical waters. Its presence has been related to toxic outbreaks affecting humans with symptoms such as coughing, fever, rhinorrhoea, mild breathing problems and skin irritation. Since the first report in the Basque Country in 2007, its abundance and distribution have grown to such an extent that, in the summer of 2021, toxic blooms of these dinoflagellates were reported for the first time in the Bay of Biscay, forcing the closure of some beaches. Consequently, a monitoring program was developed to know the current dynamics of this genus in Basque waters. The sampling took place at six sites along the Basque coast, and was carried out on a monthly basis throughout two whole years, with an intensification in summer, when it was fortnightly. The study consisted of the microscopic analysis of samples from the water column and macrophytes (Utermöhl method), the characterization of environmental variables (temperature, salinity, pH, oxygen and nutrients) and molecular analyses (rDNA barcoding) to identify the different *Ostreopsis* species.

In both sample types, the months with greater *Ostreopsis* abundances were July, August and September. Regarding the counts from water samples, although some peaks took place in Mutriku and Zumaia, the maxima were most frequently observed in San Sebastian, with a maximum value of  $1.33 \cdot 10^5$  cells/L. In the case of macrophyte samples, indicators of *Ostreopsis* reservoirs, the pattern was similar, occurring some peaks in Mutriku, Zarautz and Hondarribia, but mostly in San Sebastian, where the maximum value was recorded ( $1.86 \cdot 10^6$  cells/g) on *Centroceras clavulatum*. Molecular analyses confirmed the presence of both *Ostreopsis* cf. *ovata* and *Ostreopsis* cf. *siamensis* in San Sebastian. These results aim to help to understand the behaviour of this microalga and prevent future harmful events.

##### **4.14.P-Th450 High-Frequency Monitoring of Lake Plankton Abundances**

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Plankton is relevant due to its crucial role in ecosystem processes, such as carbon fixation and oxygen production, as well as its significance in aquatic food webs and the monitoring and forecasting of harmful algal blooms. Algal blooms are of great importance due to their significant impact on aquatic ecosystems and human activities. These blooms can lead to the depletion of oxygen in water bodies, causing harm to fish and other aquatic organisms, and can also produce toxins that pose a threat to human health when consumed through contaminated water or seafood.

In order to explain, and possibly even predict, algal blooms, we need a deep understanding of the community dynamics regulating the presence of (phyto)plankton. Ideally, this would require real-time access to the abundances of all the species present in an aquatic ecosystem.

We approached such an endeavor by installing an underwater plankton camera at 3m depth, in a medium-sized Swiss lake.



This camera records pictures of plankton at a 6 s frequency, and can detect objects ranging from 10s of micrometers to several millimeters in size. We use deep learning classifiers to identify the objects in the images. These classifiers have very high performances, which allow us to track the abundances of each taxonomic unit in real time. This methodology gives us access to real-time data on plankton abundance, biomass, and community composition, allowing for immediate detection and response to changes in the ecosystem, such as harmful algal blooms or shifts in plankton populations.

#### **4.14.P-Th451 Seasonal variability of tetrodotoxin and analogues in trumpet shell, *Charonia Lampas***

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Tetrodotoxin (TTX) is a potent neurotoxin responsible for numerous human poisonings, the majority of which have been linked to pufferfish consumption and were until the last decade restricted to Japan and other Southeast Asian countries. As of 2005, TTX-bearing species started to appear in European coastal countries. The only known case of human TTX poisoning in Europe has been linked to the consumption of trumpet shell *Charonia lampas* purchased in a market in Malaga (south of Spain) but presumed to have been caught off the Algarve coast (south of Portugal). To date, TTX is still not regulated or monitored in Europe. However, to protect public health, the European Food Safety Authority (EFSA) recommends a maximum limit of 44 g TTX equivalent (eq)/kg of shellfish meat.

To assess consumer risk from TTX-contaminated trumpet shell, we studied the seasonal variability of TTX and analogues concentrations in edible and non-edible fractions of trumpet shell specimens caught off the Algarve coast. TTX was found in varying concentrations, most of which were above EFSA recommended maximum limit, in the non-edible parts of 16 of 25 specimens collected between 2021 and 2022. No TTX was detected in the edible parts of any of the specimens analysed. However, TTX analogues were present in the majority of both edible and non-edible parts. These results suggest that although thorough evisceration may lower the amount of TTX consumed, it may not be sufficient to ensure consumer safety.

#### **4.14.P-Th452 Enhanced Control of Biological Contaminants and Biotoxins in Shellfish Aquaculture through UV-LED Technology**

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Effective control of biological contaminants and associated biotoxins is critical in shellfish cultivation, ensuring both food safety and ecological sustainability. This necessitates the integration of effective treatment systems. This study explores an emerging UV radiation technology employing LED diodes, coupled with widely utilized aquaculture oxidizing agents, namely H<sub>2</sub>O<sub>2</sub> and O<sub>3</sub>. The synergistic application of UV-LED technology and oxidative agents is designed to trigger photochemical reactions, able to effectively inactivate microorganisms and degrade biotoxins.

Preliminary findings indicate the UV-LED technology's capacity to effectively inactivate marine microorganisms, including certain *Vibrio* species and harmful microalgae. Nonetheless, some biotoxins exhibit resistance to these treatments, warranting advanced methodologies. Factors such as dissolved salts and other components within the aquatic matrix can influence biotoxin degradation, necessitating specific optimization of treatment protocols.

In conclusion, the integration of UV-LED technology, complemented by oxidative agents, presents a promising avenue for supporting the water-energy nexus in shellfish aquaculture. The demonstrated efficacy in inactivating marine microorganisms and harmful microalgae highlights its potential in ensuring the safety and sustainability of shellfish production systems. The need for laboratory studies is needed to guarantee the successful scaling of these processes from controlled environments to practical, large-scale applications.

#### **4.14.P-Th453 Efficient Adsorbents based on Covalent Organic Frameworks and Polymers for Biotoxins from Harmful Algal Blooms**

*Begoña Espina<sup>1</sup>, Miguel Chaves<sup>2</sup>, Tianxing Wang<sup>2</sup>, Vanesa Romero<sup>3</sup>, Soraia Fernandes<sup>2</sup>, Laura Rodriguez-Lorenzo<sup>4</sup>, Laura M. Salonen<sup>5</sup>, Tolga Zorlu<sup>2</sup> and Joana Campos Araújo<sup>2</sup>, (1)International Iberian Nanotechnology Laboratory, Portugal, (2)International Iberian Nanotechnology Laboratory, (3)Centro de Investigación Mariña, Universidade de Vigo, Spain, (4)Water Quality Group, International Iberian Nanotechnology Laboratory (INL), Portugal, (5)CINBIO, Universidade de Vigo, Department of Organic Chemistry, Spain*

Recently, covalent organic frameworks (COFs), crystalline porous materials, were shown to adsorb okadaic acid (OA) from synthetic seawater with a much higher efficiency as compared to polymeric resins. Equilibrium was reached in 60 min and maximum adsorption capacity,  $q_m$ , was calculated to be 279 mg g<sup>-1</sup>, resulting in a 200-fold increase compared to the state-of-the-art. In a follow-up study, the isolation of this powder-form adsorbent from the water matrix was improved by preparing a magnetic COF composite (mTpBD-Me<sub>2</sub>) that can be collected using an external magnetic field, which resulted in a further enhancement of the adsorption efficiency by a factor of three.

In the present study, we report a new, efficient synthesis procedure for mTpBD-Me<sub>2</sub> in one step that retains its adsorption efficiency as well as the use of TPB-DMTP COF for an even higher adsorption capacity and much faster adsorption reaching equilibrium in only 1 min.

On the other hand, herein, we report a covalent organic polymer (COP) for the efficient adsorption of saxitoxin (STX), the carboxylic acid functionalized TpPa-COOH. The adsorption being fast with equilibrium reached within 1h, and a calculated maximum adsorption capacity,  $q_m$ , of 4.92 mg g<sup>-1</sup>, making this material the best reported adsorbent for this toxin. More importantly, TpPa-COOH showed good reusability and high recovery rates for STX in natural freshwater.

We have prepared a carboxyl-functionalized TpPa-COOH COP with high adsorption efficiency towards STX, and reusable with high recovery efficiency. We have also improved the cost, time and sustainability of the production of mTpBD-Me<sub>2</sub> previously reported for extraction of OA from marine water and reported the high capacity and efficiency in OA adsorption of a TPB-DMTP COF. All those developments shows COFs/COPs and their composite's potential for biotoxins extraction and preconcentration for analytical applications.

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#### **4.14.P-Th454 Assessment of *Heterosigma akashiwo* Inactivation by UV Irradiation at Different Wavelengths**

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Ships load ballast water (ballasting) to increase maneuverability and balance. This water is a source of pollution and can generate ecological problems related to invasion of exogenous species, since it is discharged (de-ballasting) into the ocean or ports in a different area from where it was loaded. To prevent such contamination, various strategies have been proposed to minimize the number of viable organisms, such as the implementation of ballast water treatments systems as it is established by the Ballast Water Management Convention (IMO 2004). However, there are different challenges to be faced in order to improve the proposed treatments, such as the search for alternatives to traditional UV treatment based on low or medium pressure mercury lamps through the use of UV LEDs.

The present study aims to determine the effect of different wavelengths and radiation sources on a monoculture of a harmful algal bloom species, *Heterosigma akashiwo*. A low-pressure mercury lamp (254 nm) and two collimated UV LED reactors (265 nm and 275 nm) were used. Experiments were performed in duplicate and up to five doses of UVC radiation (25 - 200 mJ·cm<sup>-2</sup>) were applied. The treatment was performed using a Petri dish with a volume of 50 mL. The microalgae culture was used in exponential state, with high photosynthetic activity and a surplus nutrient medium (Guillard f/2). After applying the treatment, the culture was kept in a culture chamber at a constant temperature (24 °C) and photoperiod (16 h light: 8h dark). Fluorescence and quantum yield (QY) were measured for 14 days by using the fluorescence meter AquaPen AP-110.

Preliminary results indicate that doses of 100 and 200 mJ·cm<sup>-2</sup> reduced cell concentration by 70 and 90%, respectively, using low-pressure mercury lamps (254 nm). In the case of UVC LEDs only cell concentration was reduced (70%) with doses of 200 mJ·cm<sup>-2</sup>. Data modeling using logistic models will provide insight into how different doses affect the growth kinetics of the microalgae. In addition, the use of photosynthetic activity will be evaluated to determine the effect of UVC treatment in a rapid manner. Finally, cell damage after the application of different doses and wavelengths will be assessed.

The study of UVC treatment in monocultures of harmful microalgae is essential to develop effective control strategies, understand the impacts on the aquatic ecosystem and ensure water safety and the preservation of aquatic resources.

#### **4.14.P-Th455 Impact of Intense Agricultural Activities on Biota in a Natural Protected Area in SW Spain**

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Repeated dramatic ichthyofauna mortality events have been registered at the end of summer in 2022 and 2023 in the Estero de Domingo Rubio Protected Area (Huelva, SW Spain). Included in the Natura 2000 and Special Protection Area for Birds, this wetland of great ecological and landscape importance is bordered to the south by an industrial complex, to the west by the Tinto River estuary, to the east by the Special Area of Conservation (SAC) known as Dehesa del Estero y Montes de Moguer, and to the north by agricultural lands, which exerts strong pressure on the system due to polluted irrigation returns. In October

16<sup>th</sup> 2022, 200 tons of fish (mainly mullet but also common carps) appeared dead in the surface waters being reported toxins from cyanobacteria (*Prymnesium* sp. and *Pseudanabaena catenata*) proliferation as the cause of mortality by the regional government. With the aim of understanding the process causing the increase in cyanobacteria in the Domingo Rubio waterbodies, the system was monitored during one year. To address this issue, the determination of physico-chemical parameters (pH, electrical conductivity, oxidation-reduction potential and dissolved oxygen) as well as nutrients, organic compounds such as pesticides and metals concentrations were determined in waters of the system and its natural and anthropogenic tributaries. The appearance of the toxins that cause fish mortality was suggested to be associated with high levels of eutrophication, which, in turn, are directly associated with discharges of irrigation return flows from the surrounding agriculture that feed the system. In this context, the protection of the wetland and the implementation of sustainable agricultural practices are key measures to ensure the preservation of this system and the associated biota. Thus, cooperation between farmers, ecologists and the authorities is essential to find solutions and keep the ecological balance of this wetland and its sustainable conservation

#### **4.14.P-Th456 Cigarette Butts can Enhance Toxigenic Cyanobacteria Growth Through Fungal Parasite Infection Suppression**

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Smoked cigarette butts (CB) are one of the most common types of litter around the world, with approximately 4.5 trillion discarded annually worldwide. CBs contain more than 1000 chemicals, including metals, nicotine, and cotinine, which can leach into water threatening aquatic organisms such as cyanobacteria and fungi. Despite their prevalence, the effects of CB on freshwater organisms remain poorly understood. Chytrids are zoosporic fungi ubiquitous in freshwater environments that parasitize cyanobacteria and, thus, affect harmful bloom dynamics by delaying or suppressing their formation. Little is known about the effects of pollutants such as CB on chytrid infections and the ecological processes associated with these parasite interactions. Traditionally, ecotoxicological tests have focused on single-species systems. However, it is critical to understand how pollutants affect ecological interactions between species using multi-organism systems, like host-parasite. In this study, we investigated the effect of CB leachate at concentrations of 0.2, 2, and 10 CB L<sup>-1</sup> on the interaction between the toxigenic cyanobacterium *Planktothrix agardhii* and its chytrid parasite *Rhizophyidium megarrhizum*. Cyanobacteria growth was lower in uninfected cultures exposed to 2 and 10 CB L<sup>-1</sup> than in the control. In infected cultures, parasite fitness was adversely affected at all tested concentrations, with complete suppression of the infection at 2 and 10 CB L<sup>-1</sup>. Consequently, infection suppression promoted cyanobacteria growth at the highest tested concentration. Our results suggest that while CB hinder cyanobacteria growth, in an epidemic scenario it could foster cyanobacteria by inhibiting chytrid infections. This study sheds light on how CB leachate could favor cyanobacterial bloom formation and impact several ecological and evolutionary processes involving chytrids

#### **4.14.P-Th457 Acute Toxicity of Harmful Algae on Marine Zooplankton in the Context of Climate Change**

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The increase in human activities and their expansion into oceans and lakes have significantly disturbed aquatic environments. For instance, continuously increasing greenhouse gas emissions have resulted climate change, affecting the temperature, salinity, and pH levels in water bodies. Additionally, the overuse of agricultural fertilizers has altered the nutrient content in most of these environments. Harmful algal blooms are proliferated algae densities that are often able to produce different kinds of toxic metabolites. An increasing number of harmful algal blooms have been recorded in the last decades due to anthropogenic pressure. Some studies have even found that cyanobacteria that originally lived in freshwater environments are invading estuaries. Research has established that sudden increases in nutrients contribute to HAB occurrence and it has also linked warming temperatures to individual events. Zooplankton, vital to aquatic ecosystems, connect producers and higher-level organisms in food webs as both consumers and prey. Not much is known about the toxicity of these toxic blooms on zooplankton communities in different climate change scenarios. Therefore, in this study, we investigated the impact of climate change proxies on the toxicity of harmful algae to a harpacticoid and a calanoid copepod—*Nitokra spinipes* and *Acartia clausi*, respectively. We constructed a series of laboratory experiments with relevant temperature, salinity, pH and nutrient conditions, focusing on two microalga species the dinoflagellate *Alexandrium ostenfeldii* and the cyanobacteria *Microcystis aeruginosa*. After collecting algae, grown in these different scenarios, we assessed their toxicity in these copepods, studying mortality, swimming behaviour and ingestion rate. First, we observed variations in mortality rates, swimming speeds, and swimming distance between *N. spinipes* and *A. clausi* exposed to the same toxic algae, indicating an interspecies difference in sensitivity towards HAB blooms. Moreover, both *N. spinipes* and *A. clausi* exhibited varying sensitivities to algae grown under different conditions, suggesting that environmental factors indeed influence algal toxin production. These results provide better insights into environmental consequences of harmful algae blooms on the marine food web in the warming future.

#### **4.14.P-Th458 CYANOBACTERIA BLOOMS IN CITY PARKS: IMPLICATIONS FOR URBAN ECOLOGY IN A CLIMATE CHANGE SCENARIO**

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Cyanobacteria can produce toxins that can have negative effects on aquatic organisms, wildlife, domestic animals and humans. Lakes and ponds in urban parks, due to their location and importance raise important attention. They are frequented by people and pets, and, from an urban ecology perspective, they are important biodiversity spots and climate regulators in large cities. However, along with the increasing temperatures driven by global changes, the fact that lakes are usually shallow, tend to have stagnant water and may receive input of urban effluents makes them prone to cyanobacterial blooms to proliferate. In this work, the effects of a cyanobacterial bloom, collected from an urban park in Aveiro (Portugal), on zebrafish embryos (*Danio rerio*) were assessed, evaluating toxicity in terms of mortality, hatching, embryo development, behavior endpoints (total distance, slow and rapid movements, peripheral distance and different angle classes) and biochemical effects (oxidative stress - Glutathione-S-transferase (GST), Catalase (CAT), Glutathione Peroxidase (GPx) and Glutathione Reductase (GR); neurological damage - Acetylcholinesterase (AChE) and indicator of tissue damage - Lactate dehydrogenase (LDH)) after five days of exposure to cyanobacteria extract. The results showed that the exposure affected development (hatching and delayed development) and caused neurotoxicity, translated into hypoactive behavior, increased thigmotaxis and inhibition of ChE in zebrafish embryos. This study showed that cyanobacteria blooms in urban parks may pose risks to wildlife, pets and people that must be evaluated.

Keywords: Cyanotoxins, climate change, anthropogenic pressures, neurotoxicity, urban ecology, *Danio rerio* embryos

#### **4.14.P-Th459 Influence of Temperature on Acute and Chronic Toxicity of Marine Algal Toxins — A Case Study with Copepod *Nitokra spinipes***

**Wenxin Liu**, Ghent University (UGent), Belgium

Harmful algal blooms (HABs) – proliferated algae densities with often a toxin producing ability – have been found increasingly in both northern and southern oceans. Recent studies have established that increasing temperatures contribute to HABs occurrence. But the broader influence of climate change on these outbreaks is less well quantified. Of particular concern is the limited research on HABs toxin effects under varying temperatures, especially concerning zooplankton, a crucial component of aquatic ecosystems. They do not only consume algae but also serve as prey for organisms at higher trophic levels, hence, are pivotal in energy transfer and nutrient cycles in aquatic food webs. Therefore, we examined the impact of marine toxins on marine zooplankton in the context of climate change. We designed a series of laboratory experiments using filtered seawater to assess the toxicity of four commonly occurring algal toxins, purified and sourced from CIFGA Laboratory, on a model organism for ecotoxicological studies, *Nitokra spinipes*, exposed to three different temperatures. We evaluated acute toxicity of domoic acid and yessotoxin, respectively. Adult females were exposed to these toxins at 15, 20, and 25°C for 48 hours. EC50 values of domoic acid arranged from 11.08±3.81 to 88.51±164.89 µg/L, respectively. Also, juveniles, aged 48 to 72 hours, were exposed at 18, 20, and 22°C for the same duration. The EC50 of domoic acid in this case arranged from 65.36±10.66 to 102.76±9.52 µg/L. Mortality rates across temperatures showed no significant difference. In chronic toxicity test, larval development ratio (LDR), brood size and inter-brood time of domoic acid, yessotoxin, saxitoxin, and microcystin-LR were examined at 18, 20, and 22°C. We observed that with increasing temperatures, LDR for domoic acid increased, whereas brood size significantly decreased as toxin concentration rose. While these results are preliminary, they indicate a temperature dependent sensitivity of copepods towards toxins produced by HABs.

#### **4.14.P-Th460 Gene Expression Response in Marine Mussels Exposed to Toxic Cyanobacteria**

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The increase in cyanobacterial blooms poses a challenge to environmental and human health due to their potential ability to produce cyanotoxins. Exposure to these cyanotoxins can affect different marine trophic levels, including marine bivalves which have been shown to accumulate microcystin (MC) and cylindrospermopsin (CYN), two of the main cyanotoxins in freshwaters. The impact of cyanobacteria and cyanotoxins on this organism is of great relevance given its worldwide distribution and the fact that mussels are consumed by both humans and animals. Hence, the main objective was to analyze the expression of genes involved in xenobiotic metabolism in marine bivalves (*Mytilus galloprovincialis*) after short exposure to different cyanobacteria strains. Animals treatment consisted in the exposure to MC-producing *Microcystis aeruginosa* cells (LEGE-CC 91094) or CYN-producing *Chroococcoides ovalisporum* cells (LEGE-CC X-001). Two different control groups were used, consisting of non-feeding animals and animals feeding with the green algae mixture *Tetraselmis* and *Isochrysis*. After treatment for 3 and 5 days, samples were taken from the gills and digestive glands of the mussels and the expression of following genes were analyzed by quantitative polymerase chain reaction (qPCR): four nuclear receptor 1 family J identified in Mediterranean mussels (NRj1j $\alpha$ , NRj1j $\beta$ , NRj1j $\gamma$  and NRj1j $\delta$ ), three cytochrome P450s genes (CYP3L1, CYP3L2 and CYP3L3) and a membrane transporter gene (ATP binding cassette subfamily B member 1, ABCB1). The expression of genes was different depending on the organ analyzed, the time of exposure to algae or algae intake and the type of cyanobacteria. Thus, *C. ovalisporum* caused a significant increase in the expression of NRj1j $\alpha$ , NRj1j $\beta$ , NRj1j $\gamma$ , CYP3L1 and CYP3L3 genes in gills after 5-days of exposure in comparison to both control groups, while *M. aeruginosa* mainly produced a significant increase in ABCB1 expression in the digestive gland after both exposure periods. The results indicated that cyanobacteria may produce changes in the expression of genes involved in the xenobiotic pathways.

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Keywords: Cylindrospermopsin, Microcystin, Bivalves, Gene expression, Cyanobacteria

#### **4.14.P-Th461 Cylindrospermopsin Neurotoxicity in a Human Brain Spheroid Model**

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Cylindrospermopsin (CYN) is a cyanotoxin produced by cyanobacteria that is of increasing global concern due to its wide distribution, bioaccumulation capacity and toxic effects. CYN is mainly considered to be nephrotoxic and hepatotoxic. However, it can also induce toxicity in other organs, including the nervous system, although effects at this level are still poorly understood. Therefore, the aim of this study was to evaluate the effects of CYN on an iPSC-derived human 3D brain microphysiological system (BMPS). For this purpose, neural progenitor cells (NPC) were used to establish the BMPS model. The CRL-2097 fibroblast-derived iPSC cell line was used as neuronal progenitor cells (NPCs), and the toxicity of CYN was tested after 24 and 48 hours of exposure. CYN significantly reduced cell viability, with EC<sub>50</sub> values of  $3.07 \pm 0.38$  and  $1.63 \pm 0.05$   $\mu$ M after 24 and 48 h, respectively. The BMPS model was established and to characterise the different stages of differentiation and maturation, BMPS were harvested every two weeks for 8 weeks of differentiation. Analysis of different neuronal and glial cell specific genes was performed by quantitative RT-PCR. The cytotoxicity of CYN in the BMPS model was also evaluated after 1 week of exposure to different concentrations (0, 0.01, 0.1, 1, 2 and 5  $\mu$ M). A significant decrease in cell viability was observed after exposure to 1, 2 and 5  $\mu$ M. The EC<sub>50</sub> was  $4.26 \pm 0.77$   $\mu$ M. The effects at non-cytotoxic concentrations on the mRNA expression of different genes were evaluated by RT-PCR. The results showed a concentration-dependent decrease in the expression of B3T and GFAP, while other nervous markers (NFH, OLIG1, SYP) showed a high increase at 1  $\mu$ M. Oxidative stress related genes (HMOX, GSST, SOD1) and inflammatory genes (IL-1 $\beta$ , Nf- $\kappa$  $\beta$ , TNF- $\alpha$ ) showed a significant increase at 1  $\mu$ M CYN compared to the control group. Finally, immunohistochemical analysis are currently under way. These first results showed that the toxin could induce neurotoxicity in an *in vitro* 3D human model. However, more studies are necessary to determine the effects of CYN in the nervous system.

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#### **4.14.P-Th462 Neurotoxic Assessment of Cylindrospermopsin and Microcystin-LR Mixtures in Rat Brain**

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Anthropogenic activities and climate change are increasing the extent and frequency of cyanobacterial blooms capable of producing cyanotoxins. Cylindrospermopsin (CYN) and microcystin-LR (MC-LR) are among the most frequent cyanotoxins. Although they are considered mainly as a cytotoxin and a hepatotoxin respectively, different studies have revealed neurotoxic properties for both. Furthermore, the simultaneous presence of CYN and MC-LR has been described in nature. Therefore, the aim of this study was to evaluate the potential neurotoxicity of CYN/MC-LR combinations based on the measure of acetylcholinesterase (AChE) activity and different biomarkers of oxidative stress: lipid peroxidation (LPO) levels and superoxide dismutase (SOD) activity in brain of Wistar rats. Rats of both sexes were exposed orally to different concentrations of CYN/MC-LR (7.5 + 75, 23.7 + 237, and 75 + 750  $\mu$ g CYN + MC-LR/kg body weight). The methodology used to analyze these parameters was as follows: Ellman’s procedure with modifications for AChE activity, the thiobarbituric acid method for LPO levels and the xanthine-oxidase cytochrome C method for SOD activity. Interestingly, the results showed a greater alteration in the parameters analyzed at the intermediate dose employed (23 + 237  $\mu$ g CYN + MC-LR/kg body weight), being these alterations different depending on the gender of the animals. Specifically, in males the exposure to the intermediate dose produced a significant increase in LPO levels (1.5-fold) and in SOD activity (1.4-fold) with respect to the control, whereas in females the parameter with the greatest alteration at this dose was AChE activity, in which an inhibition of 20% of activity compared to the control group was observed. These results demonstrated that the CYN + MC-LR mixture may cause neurotoxic effects in rats after oral exposure and highlight the need for further studies focused on the neurotoxicity of these cyanotoxins.

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Keywords: Cylindrospermopsin, Microcystin, Neurotoxicity, Brain

#### **4.14.P-Th463 Investigation of Anatoxin-a Uptake and its Possible Cytotoxic Effects in Different Cell Lines**

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Anatoxin-a (ATX-a) is a cyanotoxin involved in cases of animal and human poisoning reported worldwide. This cyanotoxin is

classified as primarily neurotoxic; however, recent studies have described adverse effects on immune cells and at other levels following exposure to ATX-a. Despite this, the number of studies on the toxic effects produced by ATX-a in established cell lines is limited. Therefore, the aim of the present work is focused on the evaluation of the uptake and potential *in vitro* cytotoxicity of pure ATX-a in different established cell lines. Specifically, neuroblastoma (SH-SY5Y and N2a), liver carcinoma (HepG2), colorectal adenocarcinoma (Caco-2), and immune system (THP-1, Jurkat and L5178YTk±) cell lines were employed. ATX-a uptake by the cell lines was assessed by measuring intracellular and extracellular toxin levels using high performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) in cells exposed to 50 or 100 µg/mL ATX-a for 24 h. To evaluate cytotoxicity, the tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxyphenyl]-2-[4-sulfophenyl]-2H-tetrazolium) reduction (MTS) assay was performed after 24 h exposure of cells to pure ATX-a (0-200 µg/mL). Considering the amount of intracellular and extracellular toxin, ATX-a recoveries ranged from 61 to 104%, although ATX-a was only detected inside neuronal and immune cell lines (in a range of 1.9 and 13.1% of the total toxin added), while in Caco-2 and HepG2 cells the toxin only was detected in the extracellular fraction. Regarding to cytotoxicity, ATX-a only produced a decrease in viability in the cell lines THP-1 and Jurkat under the conditions employed, obtaining a median lethal concentration (LC<sub>50</sub>) only in the THP-1 cell line. This could be explained by the low amount of toxin present inside the cells, being THP-1 the cell line with the highest % of ATX-a absorbed. Thus, these data suggest that ATX-a may require a specific entry mechanism at the cellular level not described to date. Further studies on the absorption mechanisms of ATX-a are necessary to adequately select *in vitro* experimental models for the evaluation of its toxicity.

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Keywords: Anatoxin-a, *in vitro*, cytotoxicity, uptake, cellular viability

#### **4.14.P-Th464 Immunomodulatory Effects of Arsenic, Cadmium, and their Combinations with Cylindrospermopsin on the Human THP-1 Cell Line.**

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Cylindrospermopsin (CYN) is a cyanotoxin produced by cyanobacteria that is of increasing global concern due to its wide distribution, bioaccumulation capacity and toxic effects. Previous studies suggest an interaction between these toxins and other chemical contaminants that can be found together in nature. Therefore, the aim of this study was to evaluate the effects of arsenic (As), cadmium (Cd) and their combinations with CYN on the human THP-1 cell line. For this purpose, cytotoxicity assays were performed using the MTS assay. As and Cd significantly reduced cell viability after 24h exposure, with EC<sub>50</sub> of 46.48 ± 0.17 and 55.09 ± 4.98 µM, respectively. The interactions between these contaminants and CYN were evaluated using the isobologram method, which revealed an antagonistic relationship with the toxin in both cases. The effect of the mixtures on the differentiation of monocytes into macrophages was studied after 24 and 48h. Both compounds and the mixtures with CYN significantly reduced the percentage of differentiated cells compared to the control. The mechanisms of cell death were also investigated. The results showed a concentration-dependent increase in the number of apoptotic cells after exposure to both binary mixtures. The number of necrotic cells did not vary significantly after 48h exposure. Finally, the effect on the mRNA expression of different interleukins (IL-2, IL-6, IL-8, TNF-α and INF-γ) was studied. Cd caused a significant increase in the expression of TNF-α and INF-γ, whereas exposure to As increased the expression of IL-8 and INF-γ. Exposure to the mixtures increased the expression of IL-8 and INF-γ, whereas the expression of TNF-α was decreased by the Cd + CYN mixture compared to the control. These results suggest that mixtures of toxicants have different effects than single toxicants, which should be considered for a proper risk assessment.

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Keywords: cylindrospermopsin, arsenic, cadmium, THP-1

#### **4.15.P Water-Related Problems in the Mediterranean Ecoregion and Their Environmental, Health and Social Impacts**

##### **4.15.P-Tu461 Soil-Aquifer Treatment with a Reactive Barrier for Contaminant Removal: a Strategy for Using Treated Wastewater as an Adaptation Measure to Drought on the Mediterranean Coast of Spain**

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Addressing Mediterranean water scarcity demands innovative solutions like Managed Aquifer Recharge (MAR) through Soil-

Aquifer Treatment (SAT), effectively improving water quality by reducing Contaminants of Emerging Concern (CECs) through filtration, biodegradation, and sorption mechanisms.

Our study evaluated reactive barrier SAT systems' effectiveness in adsorbing CECs from urban Wastewater Treatment Plant effluents in Northeast Spain. Two SAT systems, S1 (100% sand, as control) and S2 (a reactive barrier composed of 50% sand, 30% organic matter, 10% biochar, 8% zeolite, 2% clay), underwent a 73-day winter recharge event. Using QueEChERS extraction and LC-HRMS analysis in positive mode processed by Compound Discoverer Software. A total of 1063 CECs were detected; 403 were found in S1, and 894 in S2, with an overlap of 234 CECs. To provide a comprehensive assessment, we introduced a variable called 'Total Area' ( $\sum$ Area CECs), representing the sum of the area associated with all CECs.

Crucially, depth influenced S2 significantly, showing two distinct groups: one at 15 cm and the other at depths of 28, 41, and 54 cm. Higher Total Area and CECs were observed in the latter, attributed to the initial sand composition in the first 15 cm. Notably, from 28 cm onward, S2 exhibited increased CEC adsorption and potential removal, possibly driven by biodegradation through biofilm microorganisms fueled by organic carbon from the barrier. This convergence in biodegradation pathways likely contributed to reduced contaminant detection. However, the system's performance was sensitive to fluctuations in infiltrating water CEC loads, complicating interpretation.

Contrarily, S1 displayed a noticeable upward trend in CECs over time, suggesting potential accumulation, despite the unclear influence of depth on the samples.

System 2 shows significant potential in removing CECs through extended biodegradation enhanced by biofilm formation and increased adsorption facilitated by the reactive barrier materials. This sustainable solution addresses water scarcity, especially in arid regions, promoting water reuse and storage. Implementing this technology could establish vital pathways for mitigating water stress, contributing significantly to resource management in vulnerable areas.

#### **4.15.P-Tu462 Identification, prioritisation and monitoring of the most relevant contaminants of emerging concern in a reclaimed water irrigation system**

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The reuse of regenerated wastewater emerges as a solution to address water scarcity, and its use for different purposes is subject to a legal regime that establishes permitted uses, basic quality criteria, and the treatments that must be applied. However, current treatments used in wastewater treatment plants (WWTPs) do not remove a wide variety of pollutants, such as pesticides, pharmaceuticals or industrial chemicals, which could represent a risk for the environmental and human health. To investigate this issue in an agricultural area where the irrigation water is a mixture of surface water and regenerated water, four locations (1. WWTP influent and 2. effluent, 3. reclaimed water discharged to the network of irrigation channels, and 4. water used downstream for irrigation) were sampled and analysed to identify the most relevant contaminants present in them with the application of a suspect screening (SS) workflow considering different parameters such as exact mass, isotopic pattern and fragmentation, and contrast of the data obtained with MS libraries. Subsequently, a prioritization of the top 25 compounds was conducted based on the semi-quantified Measured Environmental Concentrations (MEC) and the corresponding Predicted Non-Effect Concentrations (PNEC) in freshwater. Finally, a novel target method was developed and used to investigate the occurrence of these contaminants in the irrigation network of the agricultural area under study along one year and a final compound prioritization based on the parameters Frequency of Exceedance (FoE) and Extent of Exceedance (EoE) was performed. Galaxolidone, O-desmethyl-venlafaxine and venlafaxine appear as the compounds of highest concern.

The SS showed a total of 158 contaminants present in at least one of the samples. The site-specific priority pollutants with the highest risk quotients were the metabolite O-Desmethyl Venlafaxine and its parent compound Venlafaxine, the personal care product Galaxolidone, plus others from various other categories. The year-round monitoring at different points of the network of irrigation channels also pointed to these compounds as those showing the highest concern.

#### **4.15.P-Tu463 Role of Plastic Fragments in increasing permissiveness of E. coli towards Plasmids harboring Antibiotic Resistance Genes**

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Plastic pollution in aquatic ecosystems provides a new 'hotspot' for the colonization of bacteria, accelerating the biofilm formation and exchange of genetic material. In this work, we determined the ability of bacteria to acquire plasmid DNA from an extracellular environment under laboratory conditions. The plasmid transformation frequency was tested by setting up a single species microcosm, in which different strains of *E. coli* individually were monitored to uptake plasmid in presence and absence of plastic fragments. In total, four plastic polymers and four incubation conditions were analyzed to go deeper insight into the transformation process, and the results were confirmed by using molecular tools, and colonization ability of strains was monitored by using LIVE/DEAD™ BacLight™ Bacterial viability kit under the confocal microscope. Results illustrated

that when all three ingredients (microorganisms, plasmids and plastic fragments) were incubated simultaneously, bacterial permissiveness towards plasmid was remarkably increased. Different polymers of plastic have different ability to adhere bacteria, hence Polypropylene and Polyethylene were proven to be the most efficient plastic polymers for allowing bacteria to colonize as compared to the polyethylene terephthalate and polystyrene. The microcosm models are promising tools to mimic natural events to understand the dissemination of Antibiotic Resistant genes via HGT. Our results depict the risk of plastic pollution present in soil and water environments in promoting the distribution and spread of plasmid DNAs and their associated ARGs.

#### **4.15.P-Tu464 Bioactive Contaminants along Tyrrhenian coastal areas and their bioaccumulation in the Mediterranean endemic seagrass *Posidonia oceanica***

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Various anthropogenic activities affect the Mediterranean ecoregion, including intense cargo traffic, cruises, beach tourism, and port and industries. This basin is also subjected to significant riverine runoff transporting contaminants from cities and rural areas. The combination of these pressures and its peculiar characteristics, such as being a relatively shallow and semi-enclosed basin with no tides and weak coastal currents, makes the Mediterranean Sea an accumulation sink of several pollutants. Among contaminants, pharmaceutical and personal care products (PPCPs) and phenolic endocrine-disrupting compounds (PEDCs), have gained considerable attention for their designed bioactivity, and mutagenic and/or carcinogenic properties for organisms, causing a potential risk to ecosystem and human health. Despite this, studies reporting the occurrence and concentration of these pollutants in the Mediterranean marine ecosystems are still scarce and incomplete. In this context, the present work aims to evaluate the presence of selected PPCPs and PEDCs in two Italian coastal areas with different degrees of anthropogenic pressure. The first one is the Marine Protected Area of Rome Municipality ‘Secche di Tor Paterno’, located in the Central Tyrrhenian Sea, off the coasts of the Lazio region (Italy) few miles southern from the River Tiber mouth. The river flows through the city of Rome, and during flood events, its estuary causes low water transparency and inputs of anthropogenic contaminants into the coastal seawater. The second study area is the small town of Giglio Porto (Giglio Island in the Tuscany Archipelago, Italy), a popular summer touristic destination. *Posidonia oceanica* meadows (L. Delile 1813) develop on seabed shallower than 40 m in both areas. Surface seawater and *P. oceanica* (rhizomes and leaves) were collected to investigate the occurrence of pollutants and to evaluate the potential application of the seagrass as a bioindicator of PEDCs and PPCPs contamination. Based on the measured concentrations of these bioactive pollutants in seawater, an ecological risk evaluation was also performed. PPCPs were found at higher concentrations than PEDCs in the study areas and bioaccumulated in *P. oceanica*, suggesting that this plant can be a suitable bioindicator of organic contamination. The risk analysis indicated that the selected contaminants can pose a high risk to the marine ecosystem.

#### **4.15.P-Tu465 Exploring the occurrence of emerging contaminants in a coastal environment: the Mar Menor lagoon – Campo de Cartagena aquifer case study (SE Spain)**

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Coastal aquifers are important sources for water supply both for humans and for the environment. They are relevant groundwater-associated ecosystems offering valuable ecosystem services, and their performance can be influenced by groundwater dynamics. Meanwhile, groundwater quality is affected by land use, especially by agricultural, residential, industrial, and mining uses. These pressures lead to the presence of emerging contaminants (ECs) such as pesticides, pharmaceuticals (PhACs), and industrial chemicals, among others. The Mar Menor lagoon and its related aquifer of Campo de Cartagena (SE Spain) is a paradigmatic example of coastal lagoons where agriculture, urban development and mining use has produced an impact of huge hydrogeological, ecological, and socio-economic implications.

This study evaluates the occurrence of ECs, including PhACs, and some of their transformation products (TPs) and metabolites, endocrine disrupting compounds (EDCs) and pesticides in shallow groundwater discharging to the Mar Menor lagoon through the submerged beach. This exploratory assessment aims to expand the understanding on the occurrence of ECs in coastal ecosystems in vulnerable and hot-spot areas of contamination. This survey is part of the Spanish national project *REMEDiate*, which aims at assessing the viability of inducing in-situ groundwater denitrification in coastal aquifers discharging to lagoons, as a complementary tool to reduce nutrient and ECs input.

The survey was conducted in April 2023, including 37 sea and groundwater samples that discharge onto the lagoon. 13 widely used pesticides, 45 PhACs, 9 TPs and metabolites, and 32 EDCs were screened for using Ultra-High-Performance Liquid Chromatography (UHPLC) coupled to tandem mass spectrometry. Several ECs were identified, including the pesticides atrazine, simazine, carbendazim, terbuthylazine and irgarol, the PhACs hydrochlorothiazide, valsartan, ibuprofen,



acetaminophen, erythromycin, sulfamethoxazole, enrofloxacin, flumequine, clarithromycin, clindamycin, carbamazepine, citalopram, venlafaxine, the TPs epoxy carbamazepine and metoprolol acid, and EDCs like methylparabens.

These results characterize the extent of ECs occurrence in the shallow groundwater discharging to the Mar Menor from the Campo de Cartagena coastal aquifer, which is crucial for understanding the potential environmental impact and act as a support to targeted remediation efforts.

#### **4.15.P-Tu466 First Assessment of Microplastic Contamination in Wild Specimens of Gelatinous Zooplankton From the Northern Adriatic Sea (Italy)**

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The Mediterranean Sea is one of the most affected regions in the world by the accumulation of microplastics (MPs; < 1 mm). Due to their small size, MPs are bioavailable for a wide range of marine organisms, leading to significant negative effects to marine biodiversity. Gelatinous zooplankton is abundant and widely distributed in the Mediterranean Sea, being a major food source for secondary consumers and providing important but overlooked ecosystem services in coastal areas. Recently, this group of marine organisms has been reported as able to internalize marine litter. However, information on how such interaction occurs is still limited. The present study aims to evaluate the MP content in key Mediterranean gelatinous zooplankton, focusing on both Scyphozoans and Ctenophores species.

Specimens of *Rhizostoma pulmo*, *Cotylorhiza tuberculata* and *Mnemiopsis leidyi* were hand-collected using a cotton net between August and September 2023 along coastal area of the northern Adriatic Sea (Italy). Field blanks were placed to quantify contamination from the operators and the environment, and surface seawater samples were collected to monitor background MP concentration. In each site, a *visual census* of floating litter was also performed. Once collected, samples were stored at  $-20^{\circ}\text{C}$  individually or as pools. To extract and detect ingested MPs from samples, a suitable enzymatic and thermo-oxidative treatment was adopted, followed by a flotation step using the low cost and environmentally friendly canola oil. Then, MPs were concentrated onto glass fiber filters, counted and classified based on shape, colour and size, *via* optical digital microscope. Sub-samples were then analysed by  $\mu$ Raman spectroscopy to identify the different MP polymers.

The recovery-effectiveness of the extraction method was validated by spiking samples with a known number of MP fragments, reaching acceptable recovery rate ( $83.3 \pm 7.6\%$  for polyvinyl chloride and  $88.3 \pm 12.6\%$  polystyrene). Scanning Electron Microscopy analysis showed that the reagents used in the extraction protocol caused no detrimental impacts to the reference polymers tested, nor changes in particle weight, size and colour. Overall, the results presented on MP intake in wild gelatinous zooplankton populations will provide insights into the use of these organisms as bioindicator for MP pollution within Mediterranean pelagic communities, disclosing potential new pathways for MP entrance in marine food webs.

#### **4.15.P-Tu467 Effect of Land Use decisions on the abundance of Multi drug resistant bacteria and Antibiotic-Resistant in Bracciano Lake**

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The rise of antibiotic resistance (AR) poses a significant and escalating global challenge for public health, mainly due to the increased prevalence of bacteria resistant to routinely administered antibiotics, undermining the efficacy of antimicrobial treatment. Current study focusses on the relationship between land use decisions and the abundance of multi-drug resistant bacteria (MDR) and antibiotic resistance genes in Bracciano Lake, utilizing Geographic Information Systems (GIS) for comprehensive spatial analysis. The research integrates diverse datasets, encompassing land cover, agriculture, urbanization, wastewater discharges, and the hydrological catchment area, to understand the holistic influence of anthropogenic factors on the lake ecosystem. Spatial analyses were conducted to identify patterns within the watershed and hydrological catchment area surrounding Bracciano Lake. The results revealed that the Bracciano lake is surrounded by the residential development, industries, and tourism making it prone to anthropogenic activities, which may contribute to increased pathogen risks through potential pollutants and human activities. Considering several factors such as surface discharge, soil erosion, and groundwater flow patterns, hydrological catchment maps depicted the potential routes of the pathogens transportation from terrestrial to freshwater bodies. Obtained results emphasizes the importance of a spatially informed approach to comprehend antibiotic resistance dynamics in aquatic environments. GIS integration aids in identifying high-risk zones, informing targeted mitigation strategies. The implications extend beyond Bracciano Lake, offering insights for sustainable land management practices to safeguard water quality and public health on a broader scale. The research contributes to understanding and addressing the complex interrelationships shaping antibiotic resistance proliferation in freshwater ecosystems.

#### **4.15.P-Tu468 The Invasive Blue Crab *Callinectes Sapidus* (Rathbun, 1896) In Lazio Region (Central Tyrrhenian Sea): Updated Distribution And Possible Impacts On Shellfish Farming And Harvesting**

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The blue crab *Callinectes sapidus* (Rathbun, 1896) is native to Western Atlantic, from northern (Canada) to southern (Argentina) coasts. In its native range of distribution, the species is an important economic resource for fisheries industry. Introduced in the Mediterranean Sea in the mid of 1900s through ballast waters, the species is now spread both in eastern and western basin, representing a treat for biodiversity and affecting human activities, such as aquaculture and small-scale fisheries. The blue crab can colonize a huge variety of environments, such as coastal lagoons, saltmarshes, and freshwater habitats. It is an opportunistic predator capable to feed on fish, invertebrates (especially bivalves), detritus and macrophytes. The Lazio region, located in the Central Tyrrhenian Sea, hosts Natura 2000 sites and 19 marine aquaculture leases for shellfish farming, 9 of which are operational for mussel (*Mytilus galloprovincialis*) production. Moreover, natural bivalve banks have been registered along the regional coasts, a.g. for wedge clam (*Donax trunculus*) harvesting. Lazio also represents the second region at national level for marine fish farming production. On 2022, within the recent regional assessment of AZA (Allocated Zones for Aquaculture) carried out in the framework of the MSP (Maritime Spatial Planning) European Directive, identified a total of 354 km<sup>2</sup> suitable for shellfish farming were identified in Lazio region: approximately 68 km<sup>2</sup> with high suitability, 277 km<sup>2</sup> of medium suitability and the remaining 9 km<sup>2</sup> of low suitability. The monitoring of blue crab distribution in the region, obtained by both direct (field campaigns) and indirect records (local fishermen interviews and social media) showed that, until summer 2023, the blue crab occurrence was scattered and stable. Since then, a huge improvement of its abundance has been observed, together with new records in previously non- invaded locations. Since its voracious and aggressive predatory behaviour, the occurrence and the continuous spread of the blue crab in Lazio represent a serious risk not only for biodiversity, but also for local shellfish farming and harvesting activities. Therefore, monitoring, updating and mapping the species distribution with respect to aquaculture facilities and harvesting areas of natural banks of bivalve molluscs are fundamental to properly manage the species invasion and to avoid the huge impacts already occurred in other Italian shellfish production sites.

#### **4.15.P-Tu469 Impacts of Shipping Discharges (Including Scrubber Water) in the Northern Adriatic Sea: Exploring Current and Future Environmental Exposure Using a Modelling Approach**

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The Northern Adriatic Sea is a narrow and shallow basin, with a very short residence time (less than 3,3 months on average), and its physical properties and circulation dynamics are mainly determined by its low bathymetry, by atmospheric forcing, (e.g., wind stress and heat fluxes), tide, and by freshwater discharges mainly along the Italian coast. This leads to the presence of several different ecosystems for marine species, although they are under significant pollution pressure because this basin is surrounded by densely inhabited and highly urbanized areas. This pressure can be related to several land-based sources (e.g., effluents discharge, agricultural activities, transport of freights and people), but also shipping activities can represent a significant contribution to chemical pollution, especially near shipping lanes and ports.

In this work an exposure assessment for benzo(a)pyrene and fluoranthene accounting for both land-based sources and shipping emissions was carried out for the Northern Adriatic Sea. The modelling effort, realized within the H2020 “EMERGE” project, focused on the integration of several high-resolution predictive models to help exploring the implications of different abatement emissions techniques for shipping emissions (e.g., exhaust gas cleaning systems) for an improved management of environmental risks.

In detail, shipping-related emissions of pollutants were simulated with the STEAM (Ship Traffic Emission Assessment Model) model, based on Automatic Identification System data from 2018, as well as two different shipping development scenarios for 2050 regarding different fuel/scrubbers use options. Land-based emissions were quantified by combining daily river flow measurements with water concentrations from routinely monitoring of each tributary in 2018. The newly-developed ChemicalDrift model, a chemical transport module part of the open-source Lagrangian framework OpenDrift, was applied for these 3 scenarios using forcing data for the case study area from the SHYFEM model (ocean currents, temperature, and salinity) and Copernicus Marine Services (mixed layer depth and winds).

Results for a baseline (2018) scenarios and two future policy scenarios (in 2050) are illustrated and prove the efficacy of the proposed modelling approach to explore the implications of different shipping emissions control technologies and to identify the main sources of coastal pollution.

#### **4.15.P-Tu470 Microcosm experiments to examine the antibiotic pollution in Mediterranean inland waters based on the response of aquatic primary producers**

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Antibiotics pollution has been documented worldwide in surface waters and groundwater. They can affect negatively aquatic communities at different trophic levels, interfering with physiological processes. Primary producers, play an important role in aquatic ecosystems for the oxygen production and nitrogen fixation. Any alteration of these communities, may result in severe bottom-up effects on other organisms at higher trophic levels.

The aim of this work was to evaluate the response of primary producers, diatoms and macrophytes to Ciprofloxacin (CIP) exposure, listed in the third Watch list of Water Frame Directive WFD 2000/60/EC, through microcosm experiments.

Diatom species selected are widely distributed and representative of Mediterranean rivers: the planktonic *Cyclotella meneghiniana*; the benthonic slow moving, *Cocconeis placentula*; pedunculate and adhering to substrates, *Gomphonema parvolum* and motile *Nitzschia amphibia*. Their response to antibiotic contamination was evaluated analysing the increasing of teratological forms of their silica cell wall, called frustule.

We investigated the macrophyte's uptake capability of CIP, using the free-floating invasive species, *Lemna minuta*, widely distributed in Mediterranean slow-moving waters.

Species were exposed at three different CIP concentrations (0.1; 0.5; 1.0 mg/L) plus negative control, each tested in triplicate, in following growth conditions: temperature of  $20 \pm 1^\circ\text{C}$ , light intensity at 3000 lux (12:12 h light/dark cycle). After 30 days of exposure (diatom life cycle), diatom samples were treated to remove organic substances (with  $\text{H}_2\text{O}_2$  and HCl) and analysed at 100x microscope to investigate morphological alterations of frustule (i.e alteration in shape and outline). After 96 hours (short term exposure test) *L. minuta* growth solution samples were collected in order to analyse the remaining CIP concentrations; in addition *L. minuta* growth rate and tolerance were assessed.

Analysis are still in progress; preliminary results on *C. meneghiniana* showed an increase of 50 % teratological forms compared to the control at 0,5 and 1,0 mg/L with deformed frustule shapes, and changes in striae pattern.

The ongoing experiment on *L. minuta* has been showing no visible effect and a good tolerance to CIP concentration up to 1.0 mg/L

#### **4.15.P-Tu471 A Comprehensive Review Of Metal(Loid) Bioaccumulation In The Invasive Blue Crab *Callinectes Sapidus* (Rathbun, 1896): The Influence Of Trophic Niche And The Possible Risks For Human Consumption**

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The blue crab *Callinectes sapidus* (Rathbun, 1896) is an important fishery resource along the native Western Atlantic coasts, where the species is native. The blue crab was accidentally introduced in the Mediterranean Sea through ballast waters and now occurs in all basin, where is considered a high invasive species affecting the native biodiversity conservation, but also aquaculture and traditional fisheries. In the light of containing its spread and impacts in the Mediterranean Sea, a valuable counteraction is represented by the direct fishing for both human consumption and for animal feed production. However, the blue crab has a wide ecologic niche, is an opportunistic predator and can colonize a huge variety of environments, such as coastal lagoons, saltmarshes, and even freshwater habitats, with different contamination levels. Therefore, the bioaccumulation of contaminants in blue crab, such as metal(loid)s, should be carefully evaluated to avoid any risk for its commercialization and consumption. The present review summarized the literature data collected both in native and non-native habitats of blue crab, highlighting its capability to bioaccumulate metal(loid)s, with reference to cadmium, nickel, mercury, iron, and zinc, depending on the investigated sites. Metal(loid)s bioaccumulation profiles were tissue-specific, with main concentrations found in hepatopancreas, liver and gills, and lower concentrations in the muscle tissues. Such differences observed in non edible and edible tissues should be considered in terms of risk for human consumption. Data also showed the role of its wide and variable trophic niche: the blue crab can accumulate elements in different chemical forms and concentrations, depending on their bioavailability in the different food resources occurring in the different environments during its entire life cycle. Therefore, the traceability of blue crab fisheries should be a priority, to avoid any risk that its collection will be carried out in contaminated sites, especially in the explosive phase of its invasion, when the resource is particularly abundant. Moreover, the metal(loid) bioaccumulation profiles must be carefully monitored in the different collecting areas, to avoid any risk for consumer's safety

#### **4.15.PC Water-Related Problems in the Mediterranean Ecoregion and Their Environmental, Health and Social Impacts**

## Track 5. Life Cycle Assessment and Foot-Printing

### 5.01.A Circularity Strategies and Life Cycle Thinking: Ensuring the Way to Sustainability

#### 5.01.A.T-01 A conceptual framework to enable the implementation of circular economy strategies in support of sustainable production and consumption

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In response to the challenge of limited supply of raw materials to rapidly adopt green transitions, the European Commission (EC) aims to minimize the loss of secondary raw materials and optimize their reuse across value chains. To achieve these goals, value chain actors need to be provided with the relevant information necessary to decide on the most appropriate Circular Economy (CE) strategy to be applied to a specific product. Gathering such information along the whole value chain is a challenging endeavour. As a concrete action, the EC is preparing general requirements for the establishment of digital product passports (DPP) that should help gathering information along the value chain. This work presents a conceptual framework that identifies indicators to monitor the progress in the implementation of CE strategies.

This conceptual framework allows the identification of CE solutions to maximize the use of resources in the economy while minimizing the associated environmental, social and economic impact. This framework presents a modular structure, where each module has a specific goal and list of questions to answer. To answer the questions adequate methodologies and indicators are defined. The first version of the conceptual framework consists of the following six modules: 1. A CE-criteria module that computes the ability of a product to enter a specific CE strategy; 2. A module computing the economic, social, and environmental impacts; 3. A module gathering the needed datasets from selected background databases; 4. An uncertainty module that estimates the level of confidence of the results; 5. An optimization module that provides recommendations on the best CE strategy to apply and 6. A conformity module that guarantees the robustness of the results and provides single indicators on sustainability and circularity.

The definition of this conceptual framework is one of the first steps of a 4-year project aiming to design, implement and test an information system useable by all actors in a product value chain. This conceptual framework defines a set of criteria, indicators and parameters, essential to inform value chain actors on optimal circular strategies enhancing the availability of secondary raw materials, including critical raw materials, in the economy. The corresponding information system, integrated with DPP solutions, enables implementation, measuring and monitoring of these circular strategies.

#### 5.01.A.T-02 A Model to Assess Environmental and Economic Impacts of Waste Management in Europe

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The EU Monitoring Framework on Circular Economy includes mass-based indicators to assess the advancements towards a circular economy (CE) in EU27. Specifically for waste management, the proposed indicators are the overall recycling rate for municipal waste and the recycling rates for specific packaging waste streams. We argue that monitoring should go beyond physical mass flows by encompassing also the environmental and socio-economic impacts to understand the effects of the CE on the key dimensions of sustainability. Therefore, we propose a model that quantifies the impact of waste management at EU27 level and for each Member State in 16 environmental impact categories, internal, external and full environmental costs, as well as employment. The model is fed with EU data on municipal waste, for which a sound methodology is proposed to correct inconsistencies and ensure mass balance closure. Waste treatment technologies and processes are described with state-of-the-art data on efficiencies, consumptions and costs. Our results bring novel findings that are relevant for policy-making at national and EU level. First, we identify the inconsistencies or discrepancies in waste data reporting and propose solutions to improve reporting. We then show distance to targets at Member State level and quantify environmental impacts incurred by waste management. Last, we quantify financial and external costs, showing where the system incurs the highest benefits or costs. Our findings reveal that remarkable differences exist across EU27 in terms of environmental and economic performance in waste management, indicating the need for targeted efforts to fulfil the ambitions of the Green Deal.

#### 5.01.A.T-03 Modelling the Environmental and Socio-Economic Impacts of Existing and Novel Technologies for Textile Waste Management in the EU: Methods, Data Sources and Hotspots

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The European Commission's "Strategy for Sustainable and Circular Textiles" has as a main goal that by 2030, all textile products on the EU market should be recyclable, be made as much as possible from recycled fibres, be free from hazardous substances and be produced in an environmentally and a socially sustainable manner. While textile waste recycling is currently

in its infancy, it is unclear what sustainability benefits could be achieved from textile recycling, or how individual textile materials should be prioritised relative to these technologies. Even though many studies have used a life cycle assessment (LCA) to evaluate the environmental impacts of textile waste management, no study offers an integrated assessment of environmental and socio-economic impacts from the perspective of future technology improvements. This study aimed at providing a state-of-the-art environmental and socio-economic assessment of all key existing and emerging textile waste technology pathways for a range of 6 selected textile waste fractions. Data sources included feedback from 220+ experts within the textile sector representing 150+ stakeholder organisations. Results indicate that reuse represents the most preferred management option across the investigated textile waste streams, under both current and future framework conditions. Environmental impacts varied between impact categories and could not be represented by a single grouping. Generally, internal costs (from -22 to 2030 EUR/t under current conditions) were much larger than external costs (from -1230 to 1230 EUR/t under current conditions), thereby dominating the socio-economic results. This illustrates the importance of shadow prices for societal costs: mechanical recycling was competitive relative to incineration, but only when CO<sub>2</sub> prices were modelled as significantly higher (at 344 and 498 EUR/t) than today. The differences in impact results between the assessed textile waste materials emphasised that the compositions of textile waste fractions are a critical part of decision-making when selecting preferred management options. It is concluded that both environmental and socio-economic perspectives should be taken into consideration when making strategic decisions. Depending on the societal priorities, the most preferred solution may be different. The study provided a basis for setting future regulatory targets for textile waste management in a European context.

#### **5.01.A.T-04 How to reach an absolute sustainability and circularity in the building sector?**

*Nada Bendahmane, Mona Nassereldine and Mathilde Louërat, CSTB*

Mineral and metal resources present major environmental and socio-economic challenges, with the building sector emerging as their primary consumer. 96% of the material mass consumed in France's construction sector consists of mineral and metal resources, underscoring the urgent need for optimizing the use of these resources.

To tackle this challenge, regulatory texts referring to the circular economy are introduced in different countries. For example, in France, the Anti-Waste and Circular Economy Law was enacted in 2020. It introduces key principles for the construction sector, notably promoting reuse, encouraging the functional economy and establishing the Extended Producer Responsibility (EPR) system.

In this regard, this study aims to propose a methodology to support the building stakeholders in maximizing circular economy practices and minimizing pressure on critical natural resources.

The proposed method is inspired by the approach developed by Ryberg et al. (2018) by defining sustainable material budgets equivalent to a planetary limit for mineral and metal resources. These budgets, established at the most appropriate spatial scale for each material, are defined for the anthropogenic flows issued from reuse and for natural resources considering their criticality. These budgets are then used to estimate sustainability indicators related to the input flows of the building project.

Circularity indicators are also estimated to quantify how the studied project contributes to the circular economy through components targeted to reuse at the end of their life.

The method is structured around these following key steps:

1. Identifying the construction project's needs.
2. Estimating flows from reuse.
3. Calculating reuse material budgets assigned to the project.
4. Calculating primary material budgets assigned to the project based on their criticality.
5. Computing sustainability indicators by comparing the building needs with the assigned material budgets.
6. Computing circularity indicators at the end of life.

The proposed method can be used in the early stages of building design as well as in a prospective approach.

In this paper, the method is presented throughout a proof-of-concept case study related to the construction of building in France. This application serves to test, refine, and operationalize the method, showcasing its potential to support building stakeholders by maximizing circular economy practices and exerting minimal pressure on critical natural resources.

#### **5.01.A.T-05 Assessing the potential of exergy as a thermodynamic material and energy efficiency indicator to guide the optimisation of industrial symbiosis projects**

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To mitigate climate change and preserve resources for future generations, the economy is investing in a profound industrial transition. These transition efforts can be grouped into two broad categories: efforts to turn away from fossil-based energy

sources with greater energy efficiency and efforts to use materials more efficiently. The development of industrial symbioses in which the energy and material waste flows of one industry are managed such that they serve as inputs to complementary industries constitute a promising avenue to potentially combine both energy and material efficiencies measures. But when trade-offs arise between increasing material efficiency or energy efficiency, or between energy and resources recovery, how should these choices be optimized? A case can be made that a single indicator of resource use should guide the design of industrial symbioses. There does exist a single indicator from thermodynamics that can quantify the “quality” of both material and energy resources by linking these to the notion of work: exergy. This approach has not commonly been used for several historical and fundamental reasons. Yet it seems important to find ways of communicating what thermodynamics has to say about the increasing consumption of resources and their dispersions. Is an exergy indicator relevant and practical in guiding the optimisation of an industrial symbiosis? Our study focuses on a tire production company. Through a material and energy flow analysis, the use flows and waste flows are quantified and translated into exergy flows. We represent the loss of exergy throughout the production cycle and end-of-life transformation. A linear optimisation model then selects the technological mixes and operation parameters that minimizes the total exergy losses. We conduct a comparative analysis between optimizing the industrial symbiosis using exergy, relative to using other sustainability and efficiency indicators. In our work, we provide a unified exergy indicator for energetic and material resources as well as an optimisation tool based on constraints which represents the reality of goods-producing companies in their environmental objectives, leading them to a more sustainable ecological transition. Our other aim is to furnish information to guide the target audience on the requirements needed to use the indicator. This method can then be used in any industry that aims to improve efficiency in resource use.

### **5.01.B Circularity Strategies and Life Cycle Thinking: Ensuring the Way to Sustainability**

#### **5.01.B.T-01 The Environmental Costs of Clean Cycles: Quantitative Analysis for the Case of PVC Window Profile Recycling in Germany**

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Plastic use in buildings has been steadily increasing in the past and has led to a significant stock of plastic materials in buildings and infrastructure, with polyvinylchloride (PVC) constituting the largest share of polymers in this sector. Although little plastic is yet recycled from construction and demolition waste, effective recycling schemes exist for instance for PVC window profiles. The latter are currently challenged by the presence of legacy contaminants in end-of-life (EOL) window profiles, in particular, lead-based stabilizers, which have been phased out in virgin PVC in Europe and are subject to use restrictions in new products. The long-lived nature of PVC window profiles may therefore threaten short-term circularity targets. In this study, the current and future level of lead contamination in EOL PVC window profiles in Germany is assessed and the effect of different market, restriction, and technology scenarios is quantified in terms of environmental impacts, material circularity, and lead phase-out. A dynamic material stock and flow model for PVC on the goods layer and lead on the substance layer was developed for window profiles in Germany from 1960 to 2100. The historic model is linked to prospective scenarios with and without restrictions on lead use in new PVC products. An LCA model is being coupled with the dynamic MFA model, to estimate environmental impacts associated with the treatment of window profile waste. The potential development over time is considered in 66 scenarios, including prospective foreground and background system scenarios. The dynamic stock and flow model revealed an increase in EOL PVC window flows by a factor of 1.7 between 2020 and 2050. Whereas a restriction of Pb in new PVC frames to 0.1% by 2034 (EU 2023/923) leads to an accelerated phase out of Pb in in-use stocks (factor 5 lower concentration by 2100), it also forms a barrier to recycling and results in additional global warming impacts of 6.9 million tonnes of CO<sub>2</sub>-eq. between 2034 and 2100 compared to a non-restricted scenario. However, the assessment of scenarios on alternative treatment pathways for EOL PVC profiles, such as municipal solid waste incineration with recovery of hydrochloric acid or with carbon capture and storage, revealed that they can partially mitigate the trade-offs linked to the restriction. Comprehensive analyses are needed to identify environmentally preferable waste management concepts and to provide robust decision support.

#### **5.01.B.T-02 Bioenergy with Carbon Capture and Storage as Catalyst for Carbon-Negative Products – A Life Cycle Assessment Case Study of Olive Oil Production**

*Ángel Galán Martín, María del Mar Contreras and Eulogio Castro Galiano, University of Jaén*

The imperative for carbon neutrality and removing CO<sub>2</sub> from the atmosphere has led to a quest for resilient and sustainable production and consumption practices. In pursuit of these climate goals, Bioenergy with Carbon Capture and Storage (BECCS) stands out as a versatile solution, providing opportunities to convert biomass resources to meet the energy demands of diverse industries. The beauty of integrating BECCS into production systems lies in its potential to achieve a net-negative greenhouse gas emissions balance within the product system; in other words, it potentially allows for the production of marketable products while actively removing CO<sub>2</sub>.

This study focuses on an illustrative case of integrating BECCS into the olive oil industry. By using Life Cycle Assessment (LCA), we evaluate the environmental implications of three scenarios, conventional virgin olive oil (VOO) production and two BECCS-integrated alternatives. The study follows a cradle-to-gate approach and the functional unit was defined as the production of a one-liter bottle of VOO. The SimaPro software and ReCiPe 2016 method were employed.

The carbon footprint (CF) of the baseline scenario was +1.89 kg CO<sub>2</sub>eq per 1-liter bottle of VOO. The first BECCS-integrated scenario, which exploits only a portion of pruning residues to be energetically self-sufficient, reduced the CF by 34.46%. The second scenario that utilized all prunings generated at the olive tree cultivation stage, achieved carbon negativity up to -0.82 kg CO<sub>2</sub>eq per 1-liter bottle). However, unintended environmental effects emerged, including terrestrial acidification, freshwater eutrophication, and ecotoxicity, driven by increased fertilizer use, biomass transportation, and the CCS system. Despite these challenges, BECCS integration substantially reduced damage to human health and ecosystem quality.

Our findings contribute valuable insights for the olive oil industry, serving as a catalyst for BECCS exploration in diverse sectors beyond food systems. This research aligns with the global pursuit of climate neutrality and provides a blueprint for integrating BECCS to promote sustainability and encourage active societal engagement in climate action by delivering products with a carbon-negative footprint for consumers.

#### **5.01.B.T-03 Environmental impacts of reducing medication waste by redispensing unused oral anticancer drugs: a life cycle assessment**

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Medication dispensed to patients for at-home use frequently remains unused after treatment continuation. Unused medication, like oral anticancer drugs (OADs), can be redispensed after quality check by the pharmacy. This study assessed the environmental impact of redispensing quality-assured OADs and explored how redispensing can be environmentally improved.

Environmental impacts of redispensing OADs were determined by a cradle-to-grave life cycle assessment, based on data from a one-year multicentre trial following 1,071 Dutch patients with cancer. Process burdens of quality-assurance materials (i.e., seal bag and time-temperature indicator) were calculated, as well as environmental impacts of a sample of six OADs used to extrapolate to all OADs used in the trial. Data on active pharmaceutical ingredient (API) production was based on patent data and scaled up to industrial scale. Impacts were quantified on human health, ecosystems, and climate change. Scenario analyses on quality assurance were conducted for optimisation.

Process burdens of the base case quality assurance procedure exceeded environmental benefits, mainly due to the time-temperature indicators used to assure product quality. When quality assurance materials were used selectively for temperature-sensitive OADs (i.e., maximum storage temperature: 25°C) environmental benefits for redispensing were obtained compared to producing new OADs. By using only visual quality checks, the environmental impacts could be optimised further.

This study showed that redispensing unused OADs can be used as suitable circularity strategy to, next to being economically beneficial, reduce waste and improve environmental sustainability of (cancer) treatment after process optimisation. In the presentation, results will be shown, as well as methodological challenges in performing the LCA on pharmaceuticals and circular strategies.

#### **5.01.B.T-04 Life Cycle Assessment Case of Study: eAOP Applied to Commercial Scale Pd Recovery from Wastewater** *Gema Amaya Santos, Chemical Engineering, University College London (UCL), London, United Kingdom*

This study compares the environmental performance of four pre-oxidation technologies for Pd recovery from pharmaceutical wastewater using ion exchange. The pre-treatment technologies are: (1) H<sub>2</sub>O<sub>2</sub> addition, (2) anodic oxidation with BDD cells, (3) combined electrochemical oxidation and H<sub>2</sub>O<sub>2</sub> formation in BDD cells, and (4) sole H<sub>2</sub>O<sub>2</sub> generation in BDD cells. The reference process is incineration without Pd recovery. Life Cycle Assessment methodology is applied with a functional unit of treating 100m<sup>3</sup> pharmaceutical effluent with 95% Pd recovery. The system boundaries include pre-treatment and ion exchange. Environmental impacts of acidification, climate change, ecotoxicity, human toxicity, and land use are calculated using the E.F. 3.0 methodology. Pd recovery is more environmentally favorable than incineration, except for land use impact when using electrochemical oxidation. Optimizing electrochemical oxidation could improve efficiency and reduce land use impact. Pd recovery from wastewater using ion exchange is more environmentally beneficial than incineration without Pd recovery. The most environmentally friendly pre-treatment technology is H<sub>2</sub>O<sub>2</sub> addition, followed by anodic oxidation with BDD cells. The combined electrochemical oxidation and H<sub>2</sub>O<sub>2</sub> formation in BDD cells and sole H<sub>2</sub>O<sub>2</sub> generation in BDD cells are less environmentally friendly due to higher energy consumption. However, optimizing electrochemical oxidation could improve efficiency and reduce land use impact.

#### **5.01.B.T-05 Synergies and Lessons: On-grid and Off-grid Solar Energy Systems in Kenya and Europe**

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Solar energy is hailed as a sustainable option however, concerns about circularity and waste in solar energy systems still persist. This research delves into the on-grid and off-grid solar energy systems of Kenya and Europe, seeking to uncover

insights for circular solutions. Unlike most previous studies, the entire solar energy system, including inverters, cables, and batteries is assessed in this work. Key questions include: What is the influence of geographical and temporal factors on the life cycle assessment of solar energy systems? What are the main barriers to the transition to a circular economy for these systems, and how do these barriers vary across different regions?

This study adopts the Life Cycle Assessment (LCA) methodology to evaluate major environmental hotspots and proposing circular approaches based on considerations regarding regulatory compliance, local infrastructure, on-grid/off-grid dynamics, and socio-economic factors. A unique aspect of our approach is the emphasis on the "second life cycle" of solar components, addressing challenges in maintaining material quality during prolonged product lifespans.

In Kenya, data collection involved two months of face-to-face meetings with over 20 stakeholders, providing a complete understanding of the local scenario. European data, sourced from meetings and literature reviews, complements this procedure, ensuring a well-rounded perspective. The gathered data are then utilized to simulate current and proposed scenarios in Kenya and Europe using the EASETECH software from the Technical University of Denmark.

Preliminary results emphasize the importance of closing material cycles, especially in electronic waste management. In Kenya, a concerning trend of lower quality and durability in off-grid systems leads to increased replacements, contributing to environmental impacts. Batteries instead, a critical component, see significant refurbishment or recycling, while circular challenges emerge for motherboards, often sent abroad for recycling, highlighting the crucial importance of implementing Extended Producer Responsibility (EPR) schemes for supporting in-situ treatment. Solar panel recycling is inadequately addressed in both Kenya and Europe, with panels accumulating in storage or landfills. This poses environmental and health risks due to hazardous substances leaching into the soil.

### **5.01.P Circularity Strategies and Life Cycle Thinking: Ensuring the Way to Sustainability**

#### **5.01.P-Th467 Allocation of by-products: A multi-sector Life Cycle Assessment of low carbon steel and cement production in the United Kingdom**

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Energy intensive industries, such as the steel and cement sectors, are facing profound pressures to decarbonise through novel and innovative solutions. Within the United Kingdom (UK), the cement sector utilises supplementary cementitious materials, such as steelmaking by-products, to produce low carbon blended cements. However, the steel sector has begun a transition toward electric arc furnace steelmaking, which allows for a higher proportion of steel to be recycled. Whilst this enables a more effective use of the circular economy principle, it does not produce the co-products currently utilised in the cement sector to create lower carbon products. Life Cycle Assessment (LCA) is a standardized method to evaluate a wide range of environmental impacts throughout a product's life cycle and has become a critical lever within industrial decarbonization strategies. It has been found in existing academic literature that, currently, the allocation of by-products has not been assessed on a cross-sector level. Similarly, industrial literature would indicate that allocation procedures are chosen to minimise environmental impacts of their product. Through a novel cross-sector analysis, this research aims to conduct an LCA that can provide a holistic view on the environmental impact of BFS across the steel and cement sectors through a detailed investigation of by-product allocation procedures. Early-stage findings indicate that the allocation procedure selected has a significant effect on the environmental impact generated for a product. These results will be used to further consider how changes in UK steel production may have an impact on the production of low carbon blended cements, including assessing the effects of increased GGBFS importation due to the transition to EAF steelmaking through modelling various by-product allocation scenarios.

#### **5.01.P-Th468 Comparison of Different End-of-Life Modelling Approaches for an Environmental Life Cycle Assessment of Agrivoltaic Systems in Austria**

*Theresa Krexner, Alexander Bauer and Iris Kral, Institute of Agricultural Engineering, University of Natural Resources and Life Sciences (BOKU), Austria*

Agri-photovoltaics (agrivoltaics, APV), i.e., the parallel use of agricultural land for food/feed and electricity production using photovoltaic (PV) modules, is currently a popular research topic due to its higher land use efficiency and alternative energy source. In a previous study by the authors, the environmental impacts of two APV systems were assessed using the life cycle assessment (LCA) method and further compared with selected mono-uses of agricultural land, either for agricultural production or electricity production using PV-modules. For a holistic assessment of APV systems, a system expansion approach was used to evaluate both outputs, the agricultural goods and the PV electricity. So far, system boundaries have only been set from cradle-to-gate as to be comparable with most literature studies. However, in order to conduct a comprehensive LCA covering the whole life cycle, the end-of-life phase is now the main focus in the proposed work. Due to the many different methodological approaches for end-of-life modelling, especially for recycling, the aim of this study is to evaluate different approaches, to assess advantages and disadvantages and to demonstrate the applicability of these approaches to a multi-output product system, for which a system expansion approach has already been applied. In particular, the circular



footprint formula, the cut-off approach as implemented in the Ecoinvent database and other options, e.g., end-of-life allocation based on a closed-loop assumption will be evaluated. These approaches will mainly be applied to the steel and PV modules, as these are the most important hotspots and can be recycled in a potentially efficient way. The software openLCA v1.10.3 with the ecoinvent cut-off database 3.8 will be used; Austria is assumed as the case study region to use country specific data for e.g., steel recycling. A comparison of the results of the end-of-life modelling, but also an assessment of overall performance will be conducted. The results of the study will help to evaluate different end of life modelling options and which fit best to model APV systems.

#### **5.01.P-Th469 Environmental Impact Effect of Food Loss Reduction by Automated Ordering System**

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In recent years, there has been growing concern regarding food loss that is thrown away when it could be eaten. To reduce food loss, it is necessary to optimize distribution volume in the retail and wholesale industries, which are located further upstream. According to previous studies, optimization of purchasing by optimizing orders is estimated to be the most effective way to reduce environmental impact. However, the validity of this estimate has not been verified. Therefore, in this study, we quantified the environmental impact reduction effect when supermarket ordering is optimized by an automatic demand forecasting ordering system using primary data. The environmental impact of the following 16 items covered by the automated ordering system was quantified for each store at the production and disposal stages. The results showed that in most stores, the system contributed to reduce food loss and environmental impact. On the other hand, it was found that some stores did not fully benefit from automated ordering depending on the type of store location, regional characteristics, and specific product items.

#### **5.01.P-Th471 Identification of Environmentally Optimal Process Design for a Large-Scale Cellulose Nanocrystals Production**

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Nanocrystalline Cellulose (CNC) is a highly promising class of materials due to its favorable functional properties and its green credential as biobased material. CNC's unique properties make it suitable for a broad range of end-use applications, including pigments, packaging, electronics, medicine, etc; and there is a growing interest in scaling up its production. Today's most common method of CNC manufacturing is sulfuric acid hydrolysis of Kraft pulp. As in any other process, there exist several design options for the overall CNC production process. Recycling of sulfuric acid (as a major process input) is generally seen as a pathway for more circular and sustainable CNC production design. However, chemicals recycling processes are often energy intensive and pose trade-offs in achieving environmental benefits. Hence to ensure improved sustainability, decisions on adoption of a specific plant configuration should be based on the economic as well as the environmental data from the life cycle assessment (LCA) perspective.

In this study we establish a benchmark LCA of a conceptual large-scale CNC manufacturing process based on sulfuric acid hydrolysis. We then employ the benchmark process model to explore several plant configurations under different acid recycling scenarios. The outcomes of LCAs that incorporate energy intensive processes are highly sensitive to the type of energy mix and consequently to specific geographies of production. Our analysis of CNC production's environmental impacts for a benchmark case study in Quebec, Canada revealed the following key findings: (1) without acid recycling environmental impacts are dominated by input materials; (2) introducing circular design through acid recycling shifts environmental impacts from input materials to energy consumption: thus, complete acid recycling requires ~ 14 times more energy; (3) based on the LCA study we recommend optimizing plant configuration through partial acid recycling for a balance between materials and energy consumption. For improvement of climate change impact, regions with abundant low-carbon energy, like Quebec in Canada and Sweden, favour high acid recycling, while fossil-dominated regions benefit from limited acid recycling. When several environmental impact categories are considered, exact optimal process configuration, such as per cent acid recycling, will require multi-criteria decision analysis, considering the product specification and energy mix of the plants' location.

#### **5.01.P-Th472 Homogeneous Green Conversion of the Bio-Based Platform HMF (5-Hydroxymethylfurfural) into BHMF (2,5-Bis(hydroxymethyl)furan): Synthesis and LCA (Life Cycle Assessment)**

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The global imperative to shift away from fossil-based products has highlighted the pivotal role of biorefinery in harnessing biomasses as a source for alternative chemicals, prominently featuring 5-hydroxymethylfurfural (HMF) among its derivatives. HMF, extracted from biomasses, emerges as a versatile compound capable of undergoing several transformations.

This compound's intriguing structure opens pathways for various chemical alterations. Such transformations pave the way for HMF and its derivatives to replace conventional fossil-based chemicals across an array of applications, spanning polymer production, pharmaceuticals and biofuels.

Of particular interest is the reduction of HMF, leading to the production of 2,5-bishydroxymethylfuran (BHMF), an important compound for the polymer and flavor industries. However, the conventional production of BHMF typically involves heterogeneous systems operating at high temperatures and pressures. A homogeneous catalyst that has led to excellent results is the Shvo system, an organo-ruthenium compound<sup>1</sup>.

However, the environmental impact of the catalyst (in particular of the Ru) cannot be overlooked. The extraction, processing, and disposal of ruthenium present considerable ecological concerns. This aspect highlights the ongoing need for exploration and development of new systems that balance effectiveness with reduced environmental impact to further enhance the environmental sustainability of bio-based chemical production.

In this study, the synthesis of BHMF was explored using an iron-based catalyst, presenting comparable outcomes to the Shvo catalyst synthesis while prioritizing sustainability. Iron was chosen for its accessibility and reduced environmental impact.

To evaluate the environmental implications of this reaction, a life cycle analysis (LCA) was conducted across multiple impact categories. As the technology remains in its early stages at TRL 4, an environmental assessment was carried out to represent both laboratory conditions and a prospective analysis for potential industrial application, known as an Ex-Ante LCA. This type of study anticipates the future maturity of lower scale technologies studied at present. The objective was to calculate the overall environmental impacts, considering not only climate change but various other impact categories. Additionally, an economic feasibility assessment and a preliminary Life Cycle Costing (LCC) were performed.

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#### **5.01.P-Th473 Eco-design Approach for the Steel Circularity: a Case Study of a Steel Radiator Manufacturing**

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The production of steel as a raw material has a significant environmental footprint stemming from the extensive utilization of primary resources, substantial waste generation, emissions, and a high energy demand. Therefore, an eco-design approach supported by the Life Cycle Assessment (LCA) methodology is necessary to introduce the idea of sustainability and underline the importance of using secondary or recycled material within a concept of resource circularity. The eco-design process can be applied either during the selection of primary materials or in a more comprehensive manner that encompasses the entire production chain. This may involve considerations such as enhancing energy efficiency, minimizing waste, and optimizing production sourcing. The current study aims to perform an LCA study for the production of a steel radiator and compare different eco-design approaches in order to understand their benefits. The circularity approach involves the creation of three scenarios featuring varying percentages of scrap steels. Additionally, a fourth scenario has been developed to examine the production of "carbon-free" steel, aiming to introduce a sustainability concept encompassing the entire supply chain (cradle to gate). The production of "carbon-free" steel involves modifying the energy resources employed throughout the entire production stages, from the extraction of raw materials to the alloy processing. This adaptation included the utilization of green technologies, such as green hydrogen and electricity sourced from renewable sources, as substitutes for traditional fossil fuels like coal, diesel, and natural gas. The LCA study was conducted with *SimaPro 9.5* software and data from the *Ecoinvent 3.9* database. The environmental impacts are assessed according to the EN 15804 method. The findings highlight the climate change indicator as critically sensitive in steel production. To mitigate the overall environmental impact, employing the "carbon-free" steel provides notably positive results, achieving a reduction of over 50% in impact. These study outcomes can be leveraged as valuable insights or serve as a foundation for subsequent investigations within the environmental labeling domain, fostering stakeholder awareness of the enhanced value associated with products embracing an eco-design approach.

#### **5.01.P-Th474 Environmental Assessment of Steel Slags Ball Milling Carbonation a Carbon Capture, Utilization and Storage Material**

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The steel industry is one of the most concentrated anthropogenic point sources on the planet. In addition to the emissions, steel slags are also generated as a by-product of the manufacturing process at between 15 to 25% of the crude steel mass produced. Given the current emphasis towards a carbon reduction of 80-95% of the steel value chain by 2050 as part of the European Green Deal on Steel, there is an urgent need to adopt circular economy strategies to the current value chain. Presently, the latest research pointed to the potential to generate stable carbonates and silicates from steel slags via mineral carbonation. The process utilizes the carbon dioxide in the flue gases to form stable carbonates and silicate layers in the slags for permanent carbon sequestration. Mineral carbonation could be a potential solution to generate values from both the carbon emissions and the steel slags, thereby, enhancing a circular economy approach to waste management within the steel manufacturing industry. Subsequently, carbonated slags can be designated as a carbon capture, utilization and storage (CCUS) material as the carbonated steel slags could be applied in the construction and agricultural sectors. Ball milling carbonation is a recent technology which employs the use of milling beads to reduce the size of the steel slags which consequently increases the surface area for the carbon dioxide to be sequestered onto the slags. Nonetheless, it is important to determine whether the benefits of carbon dioxide sequestration outweigh the emissions resulting from the carbonation process. To this end, this study

has an objective to perform a life cycle assessment on the ball milling steel slags carbonation in order to (1) evaluate the environmental impacts of the ball milling carbonation, (2) identify the process hotspots for further future optimization and (3) calculate the optimum tradeoff point between the amount of carbon dioxide sequestered and the emissions during the ball milling carbonation process. The results obtained from this study can aid in the promotion and adoption of mineral carbonation of steel slags as a CCUS. Furthermore, mineral carbonation can also improve the industrial symbiosis between different manufacturing processes within and around the steel industry in such a way that promotes a circular economy value chain as well as advancing the transition towards a more environmentally sustainable steel manufacturing value chain.

#### **5.01.P-Th475 State of the art on the environmental sustainability of aluminum foundries based on Life Cycle**

**Assessment: a systematic literature review.**

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The metals sector represents one of the most impactful industries from an environmental point of view. Overall, metals production determines around 7% of the global energy demand and 8-10% of global greenhouse gas (GHG) emissions. In this sector, virgin aluminum production represents the most energy-intensive activity; for instance, the production of each ton of aluminum requires about eight times more energy than steel and iron. Not surprisingly, aluminum production is therefore responsible for about 3% of global GHG emissions. In addition, several hazardous wastes and contaminants, such as the so-called “red mud”, are produced by aluminum foundries.

On the other hand, recycling scraps can cut the energy demanded by aluminum production by 90%, but only a third of the aluminum is currently produced from secondary sources. Furthermore, secondary aluminum production and refining potentially imply relevant environmental burdens related to pollutants in off-gases, dust, slags, and dross. These issues can be, to some extent, turned into opportunities by recovering valuable materials from the by-products of both the primary and secondary aluminum industry. Moreover, innovative technologies like inert anodes are investigated by researchers to mitigate aluminum environmental burdens.

Therefore, depending on the materials' circularity and the technologies employed in metal foundries, the environmental impacts of aluminum products can be highly variable and time-dependent. Furthermore, a variety of methodological approaches and assumptions in Life Cycle Assessment (LCA) are applied in literature, especially concerning end-of-life modeling, thus affecting the variability of the results. In light of the evolution in technologies and LCA methodology, significant changes can be observed in scientific literature since the most recent review in the field was published in 2012.

This study highlights the main outcomes of a systematic literature review that is focused on the aluminum industry and LCA in a circular economy context, also remarking on perspectives and research directions in the field. This study is carried out under the framework of the “GRINS - Growing Resilient, INclusive and Sustainable” project, founded by the “NextGenerationEU” program and by the Italian Ministry of Education, University and Research. According to the aim of the GRINS project, the LCA publications related to the Italian aluminum industry are under the spotlight of our literature review.

#### **5.01.P-Th476 Leveraging Life Cycle Thinking to Select Sustainable Circular Materials for Highly Demanded and High-Performance Grout Production**

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To ensure a sustainable future, the construction sector is moving towards a circular economy, offering a promising pathway to limit environmental impacts while cultivating economic growth. C-GROUT research project under the ‘REMIND’ portfolio (Reverse Mineral Industry in Wallonia), focuses on assessing the environmental impact, performance, and feasibility of structural concrete grouts for energy market applications, where the product must withstand hard conditions. A critical aspect in this context is ensuring circularity by designing products that are safe, long-lasting, and circular. The assessment of specialized concretes in this sector uses life cycle thinking to advance circular material strategies.

The main challenge of this study is integrating secondary mineral materials available in Wallonia (Belgium), such as quarry fines, glass calcine, and various local mineral waste products, into the formulation of low, high, and ultra-high performance concrete grouts. The investigation assesses these materials during the manufacturing process, looking for ways to reduce natural resource consumption and landfill deposition to achieve equal or superior properties while having minimal environmental impacts.

Industrial partners of the project are set to conduct standardized tests to validate the technical efficiency, with the University of Liege lending its support by employing a life cycle thinking approach. This collaboration aims to unveil the environmental benefits of the resulting materials. Life Cycle Assessment (LCA) method from cradle to grave, following EN15804+A2 standard specifically, will stand as a decision-making tool to support eco-design and facilitate strategic alternative choices. Furthermore, an upscale strategy will be adapted to the potential market of the region.

The strategy is expected to result in new energy and resource efficient products, and business opportunities that will shape Wallonia's economic landscape.

This study endorses circular economy's principles, emphasizing the integration of wastes as a resource, mindful material sourcing, and creating innovative business strategies. The research highlights the significance of robust sustainability assessments that will direct the business toward products that are cost competitive. Furthermore, these new construction formulations with their minimal environmental impact point the way for future initiatives that will make a significant impact on the environment, society, and economy.

#### **5.01.P-Th477 MaIsoVi Project: Vacuum Insulation Materials, Innovative Approach for Windows in Construction and Renovation**

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The building sector is responsible for almost 36% of CO<sub>2</sub> emissions in Europe and 40% of energy consumption. This underlines the need to develop new solutions to support the strategy of reducing the energy needs of housing.

The objective of the MaIsoVi project (GreenWin n°8653) is to offer the construction and renovation market for the residential and commercial (tertiary) segment:

- A vacuum glazing (tempered FINEO) demonstrating the same or better energy efficiency than triple glazing but with the same weight as double glazing and the thickness of single glazing.
- A wooden window equipped with this FINEO and using a minimum of materials while guaranteeing the best performance over a maximum extended life span.

The implementation processes and the design of these products will be optimized to obtain a lower environmental footprint than the state of the art (triple glazed wooden window). The life cycle assessment (LCA) of the products and their processing is conducted during all the steps of the project to ensure the completion of this objective.

The tempered FINEO glazing is produced in the Walloon region at AGC site in Lodelinsart. The wooden frame is manufactured at Menuiserie Riche in Mariembourg, and the FINEO window will be marketed in Belgium, a large part of France and neighbouring countries. Two research centers, Materia Nova and Cenaero, support the development and characterization of the new products, and Liège University is in charge of the LCA.

The first step of the LCA is the specific modelling of the woods based on both primary and background data, and from this, the evaluation of the footprint of the wood window especially (eco)designed for the FINEO glazing, with different wood combinations and innovative solutions for an easy repair/replacement/refurbishing. The FINEO life cycle assessment, on its side, is based on primary data. The resulting product is to be compared to the usual commercial equivalent windows with triple glazing.

This project is supported by the GreenWin Competition Clusters and subsidized by the Walloon Region (BE).

#### **5.01.P-Th478 R3PANOT: Rethinking the tile of the future of Barcelona city**

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The R3Panot project, a collaborative effort stemming from the 21st-century Panot Challenge initiated by BitHabitat and Barcelona City Council, stands as a transformative endeavor aimed at redefining the iconic pavement design that has shaped the city's aesthetic for over a century. This pioneering project, developed by a consortium comprising Escofet, Promsa, and Eurecat, seeks not only to pay homage to the historical significance of the Panot ("tile" in catalan) but also to chart a sustainable course for its future iterations, becoming a strategic reference for subsequent generations. The project is a comprehensive exploration of sustainable practices, innovative technologies, and meticulous eco-design considerations, with the overarching goal of minimizing environmental impact while ensuring the Panot's continued cultural and historical significance.

The resulting tile from the competition proposal, with dimensions of 20x20x4.5cm, symbolize the culmination. Key among these is the use of low-emission cement formulated with steel slag, ushering in an 80% reduction in CO<sub>2</sub> footprint compared to conventional cement. Exhibiting improved mechanical resistance, the tiles additionally extend their lifetime. Furthermore, the developed tile, boast a 71% reduction in carbon footprint compared with the conventional one, includes a 30% recycled material content, mitigating the demand for primary resource extraction and an innovative recovery and repositioning system.

A significant and distinctive feature of the project is the incorporation and emphasis on the Life Cycle Assessment (LCA) as a tool for eco-design and continuous environmental improvement. Furthermore, the commitment to circularity is highlighted by the Panot's 100% recyclability at the end of its life cycle. This feature ensures that the product can be reintegrated into the production process, minimizing waste and reducing the demand for new raw materials.

In conclusion, the R3Panot project serves as a beacon of sustainability, blending tradition with innovation. By incorporating recycled content, responsible material sourcing, innovative business models, and placing a strong emphasis on LCA for continuous improvement and recyclability, the project not only reimagines the Panot for the 21st century but also sets a strategic precedent for sustainable practices in future urban design and infrastructure projects.

#### **5.01.P-Th479 Environmental performance of end-of-life scenarios of Electric Vehicles (EV) batteries following a life cycle approach**

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Energy storage is essential for the decarbonisation of the electricity grid and the transport sector. Batteries are likely to play an important role in meeting the need for short-term electricity storage in the grid and enabling electric vehicles (EVs) to store and use energy on demand. However, the use of critical raw materials (CRM) and the upstream environmental impacts of manufacturing, as well as end-of-life (EoL) management, are often cited as a drawback to the widespread use of rechargeable batteries. More efficient use of resources and efficient recycling processes are needed to reduce EU import dependency and contribute to ambitious climate targets by 2030.

It is estimated that over 80% of all energy product-related environmental impacts are determined during the design phase. Given this, special attention should be paid to the initial stages of battery design, considering the entire life cycle, with the goal of minimizing environmental repercussions. End-of-life management is of great importance and is strictly linked to the design phase. If the battery at end-of-life cannot be repaired, reused, or recycled due to an initial design constraint (e.g., disassembly problems) it will be disposable, leading to considerable environmental consequences.

In the framework of the EU Horizon Europe BATRAW project, two innovative pilot systems (TRL6-7) are being developed for sustainable end-of-life management of EV batteries, contributing to the generation of strategically important secondary CRM streams (e.g., Li, Co, Ni and Mn). In the project, an eco-design guide for battery packs is also being developed for battery pack providing recommendations at conception and manufacturing level. The BATRAW concept follows a circular approach, from collection and transport of waste battery packs, to dismantling, reparation, reuse, recycling, and sourcing of raw materials back into the EV battery manufacturing value chain. The Life Cycle Assessment methodology has been used to assess the potential environmental impacts associated to the life cycle of the BATRAW system and EoL scenarios under study (2<sup>nd</sup> life and repair of the battery pack, and recycling for the recovery of valuable materials). A comparison of the environmental profile of BATRAW with conventional production routes for valuable raw materials and traditional battery manufacturing will be presented. This project has received funding from the Horizon Europe programme under the Grant Agreement No 101058359.

#### **5.01.P-Th480 Life Cycle Assessment of Electric Traction Machine Considering Novel Recycling Processes for Permanent Magnet Circularity**

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The electrification of the fleets targets the objectives of decarbonisation of the transport sector. However, while Electric Vehicles (EV) do not emit exhaust emissions during the use phase, effects of the resource's extraction, electricity and vehicle production, maintenance and up to End-Of-Life (EoL) treatment can damage the environment. In this study, the focus is on electric traction machines (ETM). The use of strategic raw materials especially in the permanent magnets (PM) have raised concerns related to environmental performances as well as regarding the mineral resource depletion impacts. While the use of such resources helps to optimise the performance of ETM and still represents the common design for ETM, circularity strategies should help to reduce the environmental impacts of the product.

The objective of the study will be to analyse the environmental impacts of a full ETM considering a specific EoL treatment for the permanent magnet components. Such treatment consists of novel recycling processes of rare earths permanent magnets and reuse for ETM in EVs. This treatment is assessed within the context of an ETM ecodesign project called MAXIMA. Data collected for this will therefore be primary data. The idea is to understand the potential of the recycling processes considered to reduce the environmental impacts of the machine. The ETM will be used as a reference machine to which the new design will be compared during the project.

To assess the ETM, the method used will be Life Cycle Assessment (LCA), from a cradle-to-grave approach. The system boundaries will consider the ETM including the raw materials extraction, production, use phase and EoL treatment. A comparative LCA will be carried out between two scenarios: the ETM with a standard EoL treatment and the ETM including the recycling processes of PMs. The study will develop more precisely the method and allocations to consider for the systems and the approach to include recycling and circular strategy.

The results will present the impacts on all life cycle stages of the system. The goal is to show the sources of the main burdens on the environment of the ETM with a focus on the effect of improving circularity. It should give insightful information to this specific improvement in MAXIMA where the overall goal is to develop a more efficient ETM with a reduced use of resources.

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#### **5.01.P-Th481 Sustainable Design for Electric Vehicle Battery Packs: An Integrated Eco-Design Methodology**

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Advancing towards a circular economy requires a concerted effort in designing products with sustainability at their core. This research introduces an innovative methodology for eco-design applied in the MARBEL H2020 project in the design of a battery pack.

Acknowledging challenges in existing Design for Circularity (DfC) methodologies, our approach seeks to provide clarity and practicality. Rooted in the ISO 14006 standard and embracing the life cycle concept, this framework harmonizes environmental and economic considerations.

The methodology is adaptable to a wide variety of products and processes. A four-step iterative cycle, aligned with the PDSA approach, forms the core of the methodology: 1) Identification of Hotspots and Life Cycle Stages, 2) Definition and Setup of Eco-design Strategies, 3) Re-adaptation of Technological Procedures, and 4) Definition of Concrete Eco-design Actions. Stakeholder engagement ensures a comprehensive understanding of diverse perspectives, contributing to informed decision-making.

For an objective prioritization, the integrated Analytic Hierarchy Process (AHP), fosters a robust decision-making tool. All relevant stakeholders, including product designers actively participate achieving a holistic coverage of all life cycle stages. The outcomes of the application of this methodology include minimized material and resource consumption, improved functionality, enhanced environmental performance, ease of disassembly, and increased potential for recycling—all without compromising technical efficiency and considering economic aspects.

In conclusion, our work specifically addresses the application of the DfC methodology in the design of an electric vehicle battery pack, offering significant contributions:

- Identification of key environmental challenges related to LIB batteries in electric vehicles, providing the basis for targeted strategies.
- Development of a collaborative ecodesign approach, yielding 10 generic strategies and 14 specific actions tailored to the unique challenges of battery pack development.
- Provision of an initial concept design for a sustainable battery pack, encompassing all key components.

This research introduces a transferable and adaptable methodology, fostering sustainable practices in the development of battery packs and contributing to the broader goal of creating more circular and environmentally aware electric vehicles.

#### **5.01.P-Th482 To Mix or Not to Mix? Life Cycle Assessment of Critical Raw Material Recovery From Lithium-Ion Batteries by Hydrometallurgy**

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The focus of the world right now in decarbonization has disrupted global material flows, highlighting the so called critical raw materials (CRMs). Among these CRMs are materials like cobalt, lithium, manganese, nickel, etc. which are crucial in the production of batteries, the energy storage devices considered to be the key in the decarbonization of the electricity and mobility sectors. It is quite clear that since they are considered as CRMs, supply of these materials is imbalanced i.e., for the European Union (EU), most of these are supplied by other countries through processes that contradict the sustainability goals that these technologies are supposed to address. As the EU finds its footing in securing primary resources for these materials, keeping these materials in the loop through reuse and recycling becomes vital. Several researchers around the world are specially focusing on the recovery of CRMs from Li-ion batteries, a type of rechargeable battery first used on portable consumer electronics and now in electric-powered vehicles (EVs). Most of the processes that are advanced and improved are hydrometallurgical processes as they provide flexibility and are argued to have better recovery than other metallurgical approaches. However, these processes are known to be water and chemical reagent intensive due to the several steps required to ensure process efficiency. Thus, there is a need to reduce the number of separation steps and one proposed solution is to keep the CRMs together. The logic behind this is the fact that these materials are mixed to create the active materials required during battery production. The downside to this though is that the materials produced are not necessarily battery grade and would still actually need further refining, incurring additional associated impacts. This case study aims to analyze these different hydrometallurgical approaches of recovering rechargeable battery materials (e.g., Li, Ni, Co, Mn, Al, Cu, etc.) by

using simulation-based LCA in hopes of gaining insight into the best possible approach of keeping these critical metals in the loop. Specifically, the purpose is to shed light between keeping the CRMs mixed or separating them out into individual materials for reuse as battery precursors would make sense in an environmental perspective.

#### **5.01.P-Th483 Development of a Methodology for Assessing Circularity at Company Level**

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As stated by the new European Circular Economy Action Plan, circularity is an essential part of a wider transformation of industry towards climate-neutrality and long-term competitiveness. To implement circular strategies within their business models, circularity assessment is a key step for organizations to target concrete actions and pursue achievable objectives. However, standardized methodologies for assessing circularity performances at company level are still under development.

The objective of this contribution is to present an indicator-based methodology for assessing circularity at the micro level (i.e., the organization/product/service level) in the context of contaminated sites remediation which is compliant with the latest standard regarding circularity assessment (i.e., UNI/TS 11820).

The methodological development started with an in-depth analysis of the indicators included in UNI/TS 11820 and their data requirements to identify if they were suitable for their application in the field of contaminated sites remediation processes. This was followed by the setting up of a working group of experts with interdisciplinary expertise in remediation technologies design, Life Cycle Assessment, and Multi-Criteria Decision Analysis. Initially, each expert worked individually to evaluate the indicators, based on the SMART (Specific, Measurable, Achievable, Relevant and Time-bound) criteria. The outcome of this initial evaluation consisted in a preliminary selection of possibly suitable indicators and provided the necessary basis for supporting the discussion among the working group members during the second phase of the selection process. In the second phase, the working group members worked together to refine and finalize the indicators selection process. In doing so, two additional criteria have been identified: site-level applicability and respect of priority topics for remediation (use of resources, energy, water, and waste production). The identified indicators suitable for the circularity assessment of remediation processes will be presented along with the methodology to integrate them.

The developed methodology for circularity assessment of the contaminated sites remediation processes aims to be a useful tool for stakeholders and decision-makers to address problems and opportunities that can rise when implementing circular strategies in the field of contaminated sites remediation.

#### **5.01.P-Th484 Digitalisation for a Sustainable Circular Economy: the DaCapo (Digital Assets and Tools for Circular Value Chains and Manufacturing Products) Project**

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The fourth industrial (digital) revolution can drive the transformation to a more sustainable circular economy (CE). Introduction of digital technologies have the potential to reduce resource use, pollution and facilitate circular systems. Digitalisation enables transparent access to data concerning products' resource consumption and makes it possible to optimise their life cycles, which allows more efficient processes, helps minimise waste, extends the product life cycle and minimises the transaction costs, thus promoting sustainability and circularity.

The goal of the DaCapo project is to define a new set of human-centred digital tools and services to enhance the adoption of CE strategies along manufacturing value chains and products lifecycle. These tools and services will substantially improve the sustainability, efficiency, and use of imported and critical raw materials in manufacturing, driving a new paradigm of digital-enabled EU industrial sustainability and resilience.

DaCapo will be based on the following key digital developments: Digital Product Passport (DPP), Modular Digital Thread, Digital Twins (DTs) and Circular Economy Decision Support System (CE-DSS). Three different sustainability tools (Life Cycle Assessment (LCA), Life Cycle Costing (LCC), and Social Life Cycle Assessment (s-LCA)) will be used to verify that the digital tools developed in DaCapo meet the sustainability and CE objectives regarding environmental, economic and social and socio-economic impacts, respectively.

DaCapo will develop digital tools and demonstrate how digitalization could be implemented in high-impact applications in critical EU manufacturing sectors in order to contribute to sustainability and circularity, by improving their environmental footprint while achieving not relying on imported and critical raw materials. To ensure its commercial and replicability potential 3 case-studies were selected. First, Extending sustainable manufacturing and reparability approaches for aeronautic value chains based in Addictive Manufacturing (GKN Aerospace). Second, Eco-design, diagnosis and maintenance for modular mobile phones (Fairphone). Third, Elimination of bottlenecks in the warehouse industry by rethinking and renewing their logistics thanks to R-cycles in material flows for warehouse design, construction and operation (PESMEL). For this purpose, the change from a linear to a circular business model will be carried out to enhance circularity and sustainability.

### **5.01.P-Th485 Can LCA Support the Circular Economy? Evidences From Recent Scientific Literature**

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The decreasing availability of resources and the continuous production of waste is one of the main causes contributing to climate change. To overcome this problem, a series of environmental, social and economic actions are required, including moving to a Circular Economy (CE) model. The application of Life Cycle Assessment (LCA) to the CE can be useful to gain a broader view of product and organisational impacts. The aim of this paper is to investigate the application of LCA to the CE, even if it was designed to be applied from cradle to grave. This paper also wants to investigate how much scientific literature is dealing with it, with a focus in waste application.

A systematic literature review on the application of LCA to the CE was performed. Keywords 'Circular economy' and 'Life cycle assessment' were searched for in English-language scientific articles published from 2020 to 2023. To better underline the use of LCA in case of different strategies for sustainable waste management, the words 'reduce', 'reuse' and 'recycle' were searched in the titles and keywords of these articles.

Research results demonstrate great interest on combining LCA and CE: almost 1600 papers have been published in the last four years. The large number of publications shows that the use of LCA to support the research in the field of CE is increasing today and characterizes different economic sectors and different research areas. From this review, main interest of scientists about the sustainable waste management is focused on recycling, while reduce and reuse are not frequently analysed: this contradicts the international policy recommendations that push for the minimization of waste.

In conclusion, LCA seems to be considered by scientists as robust methodology to investigate the convenience of recycling options; otherwise, reduce and reuse solutions to implement the CE still appear little analysed by LCA scholars. It would be important to further investigate the interest of scientists on reduce and reuse options of CE using other impact assessment methodologies, different to the LCA. Moreover, it would be interesting to search within the scientific papers recommendations and guidelines to correctly apply the LCA in case of CE options.

### **5.01.P-Th486 Towards Cascading Use of Wood in Switzerland: Material Flow Analysis as the Basis for Circular Economy**

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Our society is facing environmental challenges such as climate change, energy availability, and resource efficiency. In this context, net-zero targets should be reached with a transition to a decarbonized society and enhancement of a bioeconomy. Forests and wood utilization are expected to contribute significantly to this goal by sequestering CO<sub>2</sub> in biomass, storing CO<sub>2</sub> in materials, and substituting energy sources. Wood, being a versatile material, plays a significant role in various industries and is a key resource for the bioeconomy, circular economy, and cascading use of material. Our study focuses on Switzerland with the aim to categorize and harmonize wood flow information for a detailed understanding the potential for a material cascade use of wood in order to support net zero targets and a circular economy. We achieve this aim by conducting a material flow analysis for the whole life cycle, from wood extraction from forest to being burned in different incineration types, of wood in Switzerland. This research addresses methodological challenges of wood as a versatile material which changes its form and composition along the life cycle. We offer a systemic view of the wood flow system, and provide insights into biomass conflicts within the wood value chain. Moreover, wood flows will be analyzed based on defined criteria (e.g., the treatment of wood) for cascading use (including reuse or recycling) in new valorization streams as a prioritized use before being burned for energy generation (as is done at the moment). The results will contribute to a bioeconomy strategy in Switzerland, while ensuring the durability, recycling, and multi-reuse of products for a circular economy, while also considering Safe and Sustainable by Design (SSbD) aspects.

### **5.01.P-Th487 Urban Mining Riches: Unveiling the Economic Value in Electronic Scrap Material for Enhanced Recycling Strategies**

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In our modern society electronic devices and their application have become heavily integrated in our everyday lives. This results in an ever-rising amount of electronic waste material as those materials are challenging to recycle due to their complex and heterogeneous composition. Especially for technology critical elements (TCEs), like Ga, Ge, In, Nb, rare earth elements, and Ta, an improvement in the life cycle is strongly demanded by the European Union to gain independence from possible supply risks. In this context, printed circuit boards (PCBs), light-emitting diodes (LEDs), or lithium batteries (LiB) gained increasing interest in the scientific but also recycling communities. The potential of environmental impacts by increasing electronic waste streams, but also the promising opportunity of urban mining are the key factors for the publicity of those three materials.



In this study end of life PCBs, LEDs and LiB were analyzed for their elemental mass fraction of 58 elements to set a standard for analytical methods for such complex matrices, thus helping the recycling industry to obtain initial estimates of the economic value of such materials.

Applying an optimized digestion protocol (microwave-assisted, 1 mL HCl, 3 mL HNO<sub>3</sub>, 1 mL HBF<sub>4</sub>) together with an improved ICP-MS/MS detection method using N<sub>2</sub>O as reaction gas, recoveries ranging from 76% to 123% for the certified reference material BAM 505a were achieved. Mass fractions of the end-of-life materials (PCBs, LEDs and LiB) ranged from 5 µg kg<sup>-1</sup> ± 17 µg kg<sup>-1</sup> (Ho) to 119 g kg<sup>-1</sup> ± 23 g kg<sup>-1</sup> (Fe) resulting in economic values between 5.71 € kg<sup>-1</sup> and 16.14 € kg<sup>-1</sup> considering all measured element mass fractions. Comparing the mass fraction to the yields of commonly mined minerals or ores, the analyzed scrap materials may possess orders of magnitude higher mass fractions of TCEs resulting in enrichment factors of up to 580 (Pd). As a result, the urban mine clearly holds high potential to generate great economic value.

#### **5.01.P-Th488 Production and use of biofertilizers from organic waste for sustainable agriculture**

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The abuse of chemical fertilizers, herbicides and other substances in conventional agriculture causes damage to the environment and nature, generating ecological and social crises. It is important to look for more sustainable alternatives such as biofertilizers where their production through semi-continuous biodigesters is efficient, economical and their assembly-handling is simple. Anaerobic digestion is a biological process that uses microorganisms to biodegrade organic matter in the absence of oxygen, produces energy in the form of biogas and has biol and biosol as by-products that can be used in agriculture as organic fertilizers. Two formulations with a fermentation period of 45 to 75 days under controlled conditions were determined. A formulation was made with various raw materials (cow excrement, molasses, ash and whey); 87% of the material that entered the biodigester was transformed into biol; which serves as a stimulant for germination and plant growth, which was proven with a phytotoxicity bioassay. The biofertilizers obtained (F1 and F2) have a neutral pH (7.77 and 8.76). Formulation 2 is the one that presents the best results in terms of macronutrients (0.04% N, 0.01% P, 0.28% K) and formulation 1 for micronutrients (21.9 ppm Fe and 3.96 ppm Mn). In both bioles, the germination index (GI) was identified through phytotoxicity tests, integrating the relative percentage of germination and the relative growth of roots. The objective of this research was to obtain an enriched biofertilizer and evaluate its agricultural use to reduce environmental impacts and promote sustainable agriculture among small producers.

#### **5.01.P-Th489 Sensitivity Analysis of a Life Cycle Assessment for Biochar in an Italian context**

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Due to its diverse applications in bioenergy production, coproducts (bio-oil and syngas), global warming mitigation, sustainable agriculture, pollutant removal, and other fields, biochar has attracted interest worldwide. Implementing soil-based biochar for carbon sequestration could not only mitigate emissions but also enhance soil quality, creating opportunities for bioenergy production. However, to comprehensively assess the environmental and energy impacts of the production cycle and validate the myriad benefits of biochar, Life Cycle Assessment (LCA) stands out as a reliable evaluation tool.

This study builds upon the work of Marzeddu and Cappelli (Marzeddu S., Cappelli A. et al., 2021) to delve into the environmental impact of an energy-biochar chain involving an Italian gasification plant. In the previous LCA, which characterized biochar used as a soil conditioner, aspects such as soil carbon sequestration, nitrous oxide emissions, fertilizer usage, and water consumption for irrigation were considered. The findings indicated that employing gasification for energy and biochar production presents an appealing strategy for mitigating environmental impacts, particularly in terms of climate change, with a net decrease of approximately -8.3·10<sup>3</sup> kg CO<sub>2</sub> eq.

However, the preceding study lacked a sensitivity analysis. Therefore, this study introduces a sensitivity analysis to consistently evaluate the environmental trade-offs associated with biochar and amended soil. Specifically, sensitivity is addressed for upstream processes by considering alternative types of woodchips. For the core process, variations in packing material are explored, and a cradle-to-grave perspective is taken by optimizing logistics, supply chain distances, and selecting the Best Available Technology (BAT) for transportation vehicles.

This research underscores strategic developments that collectively identify potential environmental trade-offs and thresholds in utilizing biochar, particularly as a soil conditioner.

#### **5.01.P-Th490 Unlocking the Technical, Economic and Environmental Implications behind a Multiproduct Biorefinery from Exhausted Olive Pomace**

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Recent advancements in bioconversion technologies have opened up exciting possibilities for extracting valuable compounds from biomass waste material. For example, the conversion of residue from olive pomace oil production has been the subject of numerous investigations, most of which concentrated on particular aspects of the procedure at the laboratory scale [1]. Filling this gap, this case study presents new insights into the valorisation of exhausted olive pomace from laboratory experimentation [1] to simulation at an industrial scale. In particular, we combined process simulation tools with Life Cycle Assessment to conduct a techno-economic and environmental analysis of a multi-biorefinery system designed for the extraction of antioxidants, lignin, and bioethanol. We defined five different scenarios which vary in the electricity source powering the processes (wind, photovoltaic, or the existing mix) and heat/cooling source either from fossil natural gas, synthetic natural gas (generated from Sabatier reaction of CO<sub>2</sub> from direct air capture and electrolytic H<sub>2</sub>) while one scenario integrates carbon capture and storage (CCS). The simulation, executed through Aspen Plus serves as a basis to generate the mass and energy balance to carry out the economic and environmental assessments using SimaPro software. The functional unit (FU) of choice is "1 kg de bioethanol + 0.487 kg de antioxidants". In terms of cost distribution, regardless of the scenario, capital expenditures (OPEX) outnumber the operational ones (CAPEX) representing around 80% of the total cost, followed by utilities (the operating heating and cooling costs) which vary greatly across the scenarios. Concerning global warming, the scenario integrating CCS (and relying on synthetic natural gas with H<sub>2</sub> generated from wind power) emerges as the most environmentally favourable option, showing the lowest carbon footprint (9.35 kg CO<sub>2</sub>eq/FU) compared to other scenarios. However, our findings also demonstrate nuanced trade-offs associated with burden shifting in these scenarios, worsening other environmental categories beyond climate. Overall our research sheds light on the broad implications of large-scale deployment of biorefineries and can guide future endeavours towards the optimization of biorefineries, fostering a resilient and eco-friendly bio-economy.

## 5.02 Innovations in LCA: Bridging Temporal Dynamics and Advancements in Inventory Data Modeling

### 5.02.T-01 FineChem2: An enhanced deep-learning model for estimating carbon footprints of chemicals

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Millions of chemicals have been designed and registered to contribute to the global efforts toward accelerating the transition to sustainable chemistry; however, their product carbon footprints (PCF) are often unknown, leaving questions on their own sustainability. This general lack of PCF data is because the data needed for comprehensive environmental analyses are generally not available at early design stages. Several predictive tools have been developed to estimate the PCF of chemicals, which are only applicable to a narrow range of common chemicals and have limited predictive ability. Here we propose FineChem 2, which is based on a Transformer framework and first-hand industry data, for accurately predicting the PCF of chemicals. Compared to previous tools, FineChem 2 demonstrates significantly better predictive power, and its applicability domains are improved by ~75% on high production volume chemicals, daily chemicals, and chemical additives in food and plastics. The better interpretability from the attention mechanism enables FineChem 2 to successfully identify PCF-intensive substructures and critical raw materials of chemicals, providing insights into design of sustainable molecules and processes. Therefore, we expect wide application of FineChem 2 for chemical PCF estimations, leading to advancements in sustainable chemistry.

### 5.02.T-02 Inventory estimation for chemical processes from the reaction stoichiometry by decision trees

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The chemical industry aims to transition toward sustainability. For this purpose, decision-makers in research and development (R&D) require process-specific Life Cycle Inventory (LCI) data. However, during early R&D, only the reaction equation is available, and the missing information on energy and utilities is often filled with average proxy values. These proxies prevent process differentiation and decision-making, as they are equal for all processes. Here, we present a method to estimate process-specific LCI data based solely on data directly derived from the reaction equation and physical property data for the chemicals involved. The method employs the machine-learning method of decision trees, which is easily accessible.

Our method is based on training decision trees to estimate the demands for electricity, steam, natural gas, cooling water, and process water, the direct emissions, and a coefficient for the raw materials global warming impacts (GWI). The trees use information about the reaction stoichiometry, such as the number of reactants and products, and property data, such as boiling points, as input data. For training, we used LCI data of 409 chemical processes for 148 organic chemicals, including platform chemicals, intermediates, and consumer chemicals. The LCI data is based on the Process Economics Program (PEP) yearbook, which offers detailed in- and output flows derived from patents, company reports, and process simulations.

The proposed decision trees estimate inventory flows for organo-chemical processes from the reaction equation alone, reaching the same accuracy as established cost estimation methods. Decision trees resolve patterns between reaction and property data with raw material and utility demands. The prediction accuracy improved for the overall GWI and each inventory information predicted, with improvements of the mean absolute error between 8 and 24 % compared to the best available proxy values. The proposed decision trees enable the automated generation of LCI data and can be used to improve background databases and inform decision-making during R&D.

An open-access toolbox is prepared for easy access. By integrating the DIPPR database, we minimized the required inputs and

increased the scalability of the tool. The decision tree LCI estimation tool can support decision-making in chemical R&D and help identify the path toward a cleaner future for the chemical industry.

### **5.02.T-03 Integrated Life Cycle Optimization of the Foreground and Background Systems using the PULPO Open-Source Python Package**

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The interpretation phase in Life Cycle Assessment (LCA) is often carried out without applying any systematic method to reduce impacts. While the life cycle optimization and TCM approaches couple LCA with optimization, they do so without attaining full integration between the foreground and background systems.

To overcome these limitations, here we introduce PULPO (Python-based User-defined Lifecycle Product Optimization), an innovative open-source Python package that integrates LCA data manipulation and optimization model creation and solution. PULPO enables the dynamic feedback between the foreground and background systems, which is achieved through the integration of complete LCI databases as backbone for the optimization model. Our goal with PULPO is to furnish the LCA community with a powerful tool that enables advanced analyses very hard to perform otherwise.

The approach is exemplified in case studies optimizing a country's power mix and global methanol supply chains. Through these case studies, PULPO demonstrated its ability to find optimal decisions across complex supply chains that minimize environmental impacts and identify efficient production pathways under various constraints (e.g., production capacity and resource availability).

The package, accessible via GitHub, facilitates a more consistent and coherent approach to life cycle optimization, assisting LCA practitioners in complex studies and providing insights out of reach for standard LCAs. Overall, PULPO provides a computational framework for environmentally conscious decision-making, that is made easily accessible through an open-source Python implementation. From a broader perspective, we intend to build bridges between the process systems and industrial ecology communities so together we can more effectively tackle the challenging problems ahead in the quest for sustainable development.

### **5.02.T-04 Result variations due to dynamic life cycle assessment compared to result variations due to sensitivity analysis on static inventory data**

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Biomass is a promising raw material for both energy and chemical production and their decarbonisation. However, calculating the impact of biomass use on climate change is still controversial, notably in terms of how to account for differences in the dynamics of carbon storage by photosynthesis and releases at the end of life (ex: incineration). Applying dynamic life cycle assessment (LCA) is one of the answers, but it requires more data and increases the complexity of the calculation. To address its relevance, we have to answer if the variation in results induced by the dynamic characterisation of the impact on climate change is significant compared with the variations induced by the uncertainty in the inventory data. This question is explored through the present case study, including the biomass production (miscanthus or wood residues), the CO<sub>2</sub> production by biomass fermentation, the conversion of the CO<sub>2</sub> into a polypropylene bag and the bag incineration with carbon capture and storage. A sensitivity analysis is performed to compare the results depending on i) the type of modelling (dynamic or static), ii) the characterization method to evaluate climate change (absolute global warming potential, AGWP, or temperature change, AGTP) and iii) one key parameter (the amount of CO<sub>2</sub> emitted by soil organic carbon change during miscanthus production). The results reveal that variations induced by the dynamic modelling compared to the uncertainty on static inventory data are significant in the case of wood residues production and more significant when using AGWP than AGTP. Using AGTP rather than AGWP to assess the impact on climate change would thus improve comparisons between systems even without distributing inventories over time. Moreover, it would be useful to develop a method for assessing the relevance of carrying out a dynamic LCA, by precisely defining the flows that will benefit from being spread out over time. This method should consider as input data some time parameters such as the duration of the inventory or the time of the first emissions. It is important to i) keep time to improve the static inventory and perform sensitivity analyses and ii) limit the loss of information due to the cut-off included in the algorithm used to calculate a complete dynamic LCA.

### **5.02.P Innovations in LCA: Bridging Temporal Dynamics and Advancements in Inventory Data Modeling**

#### **5.02.P-Mo452 The Importance of Timeframes When Determining Marginal Heat Suppliers for Informed Decision Support**

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When assessing the impacts of a decision, the timeframe for when the decision takes effect is crucial, as different marginal

heat-producing technologies will be affected in different points in time. This study will focus on how decisions, that impact heat consumption and production, can have different environmental impacts depending on when the effects of the decision take place.

For example, in 2022 the Danish government mandated that public buildings should not be heated to above 19°C. This mandate had an immediate effect on heat consumption, which affected the marginal heat suppliers at the time i.e., the production marginal. On the other hand, if it was decided to implement better insulation of public buildings, the reduction in heat consumption would take effect when the insulation has been installed, affecting the marginal heat suppliers of the future i.e., the capacity marginal. The later case is the typical approach applied in consequential LCI modelling.

As heat-producing technologies change over time, these two decisions will affect different marginal heat suppliers, and will therefore have different environmental impacts, but this is not reflected in many LCA studies. It is therefore important to develop and popularise a methodology for determining the marginal heat suppliers at different points in time. Since heating grids are localised, the marginal heat suppliers will vary between the grids. This paper therefore proposes a tool that applies a consequential modelling method for determining the marginal heat suppliers in a localised grid at a specific point in time. By using data for district heating in Denmark, the short term affected suppliers are determined based on trends for the previous five years, while the longer-term suppliers are determined by identifying likely future technologies of the specific grids, which depends on political and planning decisions.

The findings of the study will help to inform decision-makers on which decisions will be most impactful regarding reduction of environmental impacts from heat consumption, while also communicating why the timing of decisions matter.

### **5.02.P-Mo453 Regionalizing the Supply Chain in Process Life Cycle Inventory with Multiregional Input-Output Data: Ecoinvent with EXIOBASE**

*Sidi Peng and Stephan Pfister, ETH Zurich, Switzerland*

Life cycle inventory (LCI) databases like ecoinvent are essential in life cycle assessment (LCA) but lack country resolution in both the activity details and trades among regional activities in the supply chain. For instance, photovoltaic (PV) panel production has only Rest-of-world (RoW) and European (RER) datasets in ecoinvent, which consumes the same global production mix of PV cells as technosphere inputs, since it is challenging to identify the precise origins of technosphere flows for a regional activity. These global or continental activities and trade limit the utilization of the existing country processes in ecoinvent, like electricity production, and affect the accuracy and granularity of LCA results and relevant decision-making.

To address this issue, we disaggregated the global and continental unit processes in ecoinvent to country level and regionalized the trade relations among them by applying country-sector-specific consumption mixes of products generated from the multiregional input-output table (MRIO) EXIOBASE. The outcome is a consistently regionalized ecoinvent database at country level that links regionalized production impacts to regionalized consumption.

We compared the climate change impacts of all 195\*708 non-market datasets in our regionalized ecoinvent with reference datasets in the original database. Overall, manufacturing sectors show more different impacts after the supply chain regionalization, since most impacts of manufacturing activities are embedded in background supply chains.

The case study of PV panel production reveals that RoW/RER proxies in the original ecoinvent for Chinese, Japanese, and German production would cause differences of climate change impacts between -24% and +1%, compared to disaggregated country processes, mainly due to different electricity mixes used by the upstream country-specific suppliers of PV components and silicon, which get lost in the low-resolution trade data.

Our study demonstrates the significant potential of integrating better trade information and region disaggregation in LCI databases for improving the accuracy and granularity of LCA results. While the combination of two databases inherently has limitations and strengths from MRIO (low sector resolution but complete economy) and LCI data (low country and trade coverage but high process detail), it allows to have more detailed results that can help to identify processes that need further attention for proper supply chain assessment.

### **5.02.P-Mo454 Fully dynamic carbon footprint of circular biobased systems – A framework with temporal life cycle inventory database (DyPLCA) tailored for forestry and wood products cascades**

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Global concerns about climate change and its impacts induced by greenhouse gas emissions and processes make it crucial to understand its evolution over time. This is particularly relevant for biobased systems, which take up CO<sub>2</sub> during biomass growth, of which a part remains stored during the life cycle of products (considering possible recycling, reuse etc.), and there is an eventual release at the final end of life (e.g. via incineration). However, in the field of life cycle assessment (LCA) and carbon footprint analysis, a static view is still the dominant approach. Few studies have considered dynamic effects, and if so,

only by taking into account time differentiation for the foreground system. Therefore, it is only recently that a fully dynamic LCA has been applied (in this case, dynamic only implies temporal differentiation), in particular through the dynamic process-based LCA (DyPLCA) framework, tool and related temporal database. Yet, there are several aspects needing improvement, A framework has been developed to tackle these gaps, as specified in the next section.

*First*, the DyPLCA temporal database is a collection of temporal data for all ecoinvent 3.2 processes. Currently, ecoinvent 3.9 is available and contains updated information and new processes. Hence, a straightforward improvement we have done for this framework is the update of the DyPLCA database by LIST in the CALIMERO and LCA4BIO projects.

*Second*, in the respective DyPLCA database, the temporal flows of forestry systems, especially greenhouse gases, have been considered in a simplistic manner. Emission amounts and their temporal distributions are improved based on the outcomes of a flexible parametric model for a balanced account of forest carbon fluxes in LCA.

*A third gap* relates to the need to update the climate change impact assessment. INSA Toulouse has developed an advanced tool that already covers different types of effects over time and is compatible with DyPLCA outcomes. This tool and its dataset will be updated.

*The final and fourth gap* of concern is the limited tailored loop modelling for wood cascading systems in ecoinvent and DyPLCA. This is ameliorated by systematic incorporation usage processes with their durations, the possibility to adapt number of loops and the handling of recycling/reuse.

An application to two cases of the CALIMERO project is envisioned: (1) one on laminated strand lumber (LSL) & (2) the Swedish pulp and paper sector.

#### **5.02.P-Mo455 Carbon Flux Forest Model Extended to Multiple Species and Forest Management Practices: Data and Validation**

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Drawing from the work of De Rosa et al. (2017) we present our study done to extend the modelling of forest management, and the calculation of life cycle inventories for forest products, to a wide range of forest plantations and species. The use of the model is intended for the modelling of biobased products, which is then enabled and enhanced by the extension of the model and associated input data.

The model allows to generate a time-specific, dynamic flux inventory of carbon flows for a specific forest and express this impact using different climate impact metrics (both Global Warming Potential – GWP and Global Temperature Potential - GTP). However, previous published and available versions of the model only allowed the modelling of two species: Spruce and Eucalyptus, a long-rotation and a short-rotation one respectively. The present work documents the effort made to extend the input data of the model. This allows model simulations of a wide range of plant species and those commonly cultivated in Europe, as well as management practices.

We show the approach used to fill data gaps, that were substantial as no database was found to consistently report information about growth rates, rotation times, and thinning practices of different species and these had to be retrieved and aligned manually. Furthermore, we show results of a sensitivity analysis performed on the model results obtained from the simulation of several species, indicating that not surprisingly carbon content, density, and rotation time are highly sensitive parameters. A validation of the model is also presented - for selected species. Finally, we present a discussion on the correct use of the results and the linking with LCA software, specifically for the choice of environmental exchanges to be considered when modelling biogenic carbon uptake and release. We also document the improvements made to the user interface, as part of the effort in making the model user friendly and enhance interoperability by enabling import of resulting inventories in common LCA software. The work was conducted as part of the ALIGNED project ([www.alignedproject.eu](http://www.alignedproject.eu)) on harmonisation of LCA methods for the assessment of biobased products.

#### **5.02.P-Mo456 Data Quality Assessment of Aggregated Life Cycle Inventory Datasets - A Case Study on Fossil-Based and Bio-Based Plastic Food Packaging**

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In Europe, approximately 40% of food is packaged in plastics. This packaging is criticized as a symbol for make-use-dispose economy due to its rather short lifespan, its dependency on fossil fuels, and its contribution to marine litter. Environmental impacts resulting from plastic food packaging, made from both fossil-based (FB) and bio-based (BB) polymers are increasingly analyzed in LCA studies. However, the literature reveals significant variations in results up to the order of 400% for the same polymer within the same scope. To enhance the reliability of these assessments, Data Quality Assessment (DQA) plays a relevant role. Despite most of the LCA studies employing aggregated Life Cycle Inventory (LCI) datasets, in literature DQA methods for aggregated processes are not available. To fill this gap, in this study a DQA for aggregated LCI datasets is proposed and demonstrated through its application to 101 aggregated LCI datasets, extracted from Ecoinvent and GaBi

databases. The DQA method has been developed by adapting and integrating the Pedigree Matrix and the Data Quality Ranking proposed by the recently published EC Plastic LCA method. The three Data Quality Indicators (DQIs) used are Technological (TeR), Geographical (GeR), and Time-related (TiR) representativeness. To each DQI a score is assigned according to a five-point scale, where 1 is the best (corresponding to an Excellent Data Quality level), and 5 is the worst (corresponding to a Very Poor Data quality level) achievable score. The application of this method exhibits an overall positive evaluation of the selected datasets with differences between Ecoinvent and GaBi and among the three DQIs. Moreover, it highlights the role of metadata structure in adequately supporting a robust DQA. Indeed, in the absence of a common framework that defines, assesses, and provides access to data quality information, transparency must be assured by the operator in the metadata interpretation and related assumptions along the DQA process. Finally, although the proposed DQA method was developed for the plastic sector, its application can be extended to LCI aggregated datasets relevant for other sectors, materials, and products.

#### **5.02.P-Mo457 Assessing methodological choices in wood-based biorefinery LCA literature**

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Bio-refining is a process (or several processes) that converts any biomass into multiple beneficial products. Bio-refining is complex and is rapidly transforming given the increasing demand for bio-based materials particularly to augment the functionality of biomass. Modelling a bio-refinery for an LCA study is equally complex, given that it needs to address issues related to multi-functionality, choice of appropriate functional unit, identify market for feedstock, modelling of biogenic carbon, etc. We present the results of a systematic literature review to understand how these issues have been addressed in previous studies related to biorefining of woody biomass. We used the standard EN 16760:2015 for LCA of bio-based products as a framework to structure the review. The standard particularly emphasizes the need to report spatial and temporal boundaries related biomass systems, biogenic carbon flows, water use and land use. 41 studies between 2015 – 2023 which included detailed LCA models of bio-refining woody biomass were reviewed. Given the emphasis on key factors from the standard, it was surprising to find that few studies addressed the temporal aspects of biomass feedstock or water use in bio-refineries but did not account for them in the inventory. Four studies reported the biogenic carbon flows, although only two studies accounted for the temporal aspects of biogenic carbon. Most studies considered accounting for biogenic carbon ambiguous or neutral. Furthermore, most studies handle multi-functionality using economic allocation or system expansion followed by mass and energy- based allocation respectively. However, the specific use of consequential inventory modelling was limited to three studies. There was no discussion of market factors influencing the availability of biomass. Whilst there are several LCA studies on bio-refineries, this review highlights the lack of consideration given to modelling approaches that can have a key influence on the results based on source and management of biomass. Current studies lack a holistic modelling approach that could support assessment of prospective technologies. The work was conducted as a part of the ALIGNED project ([www.alignedproject.eu](http://www.alignedproject.eu)) on harmonization of LCA methods for the assessment of bio-based products.

#### **5.02.P-Mo458 Bridging scales in lithium production: From site-specific studies to a global model**

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The build-up of energy storage, such as Li-ion batteries, necessitates a significant increase in lithium supply. Brine deposits are one of the two important deposit types as they contain ~2/3 of the known lithium resources worldwide. These deposits, however, vary in chemistry and environmental conditions, requiring tailored processing techniques for optimal lithium yield. The increasing importance of lithium extraction requires a detailed life cycle assessment to account for specific impacts and avoid environmental burden shifting. While site-specific life cycle assessments have been performed, they are limited to a small number of mines because required data is scattered in the literature making it a time-consuming endeavour. There is currently no global model available that incorporates site-specific conditions to evaluate the existing and future production of lithium. Based on our previous research, the Generative Pre-trained Transformer (GPT) is used to systematically assess the processing technology reported in technical reports and feasibility studies. Data in the scientific literature are used to train GPT to classify the available information in the reports. The GPT output includes all processes from pumping the brine to the surface until the final product is produced. This data feeds into a life cycle inventory model, which allows calculating life cycle impacts of 1 kg lithium carbonate. Significant variations in climate change impacts are found, ranging from 3.2 kg CO<sub>2eq</sub>/kg lithium carbonate from Salar de Atacama to 50 kg CO<sub>2eq</sub>/kg lithium carbonate from Salton Sea, mainly due to technology choices based on brine chemistry. Technologies, such as the ones used at Salton Sea, have a higher energy demand leading to these elevated impacts compared to the sites that use conventional extractive methods. The study underscores the need for tailored assessments for current and future production of lithium. Using AI through GPT supports the systematic collection of data from various sources and also allows for detailed modelling, while assessing a broad range of current and future sites. With regard to future mining sites, this study reveals the importance of life cycle assessment at an early stage to highlight life cycle impacts – especially, reduced impacts by decarbonizing energy supply. Site-specific assessments are crucial for policy-making, offering insights beyond the scope of generic models.

#### **5.02.P-Mo459 LCA of the Italian Photovoltaic Mix for 2021 and 2030**

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Photovoltaic (PV) is one of the technologies at the base of decarbonising electricity production plans in Italy. PV industry is

experiencing rapid growth, and the technological development of PV technologies is constantly evolving. Of course the datasets available in commercial database (e.g. Ecoinvent 3.8) for the LCA modelling of Italian PV mix are neither updated nor representative of the national context; therefore, the environmental performances assessment of photovoltaic electricity production results not be reliable and underestimated.

To fill this research gap, the current study presents a detailed, updated, and representative LCA modelling of the Italian PV mix for 2021 and of that expected for 2030.

For both PV mixes, data provided by an Italian company operating on energy i.e. GSE (Gestore dei Servizi Energetici), and the annual reports of “International Technology Roadmap for Photovoltaic ” of VDMA were used to obtain the values of PV electricity production and installed PV power at the end of 2021 as well as to estimate the percentage shares of that power in function of: type of PV module as mono- and multi-crystalline silicon, thin films etc; type of PV technology; and type of PV plant configuration as ground or roof installation. The same analysis was also carried out for the period from 2021 to 2030 using data from Integrated Climate and Energy National Plan. Task 12 reports of “Photovoltaic Power Systems Programme” of International Energy Agency, together with primary data from PV industry and PV technologies developer, were the main data source for the creation of new datasets for each type of module, technology, and plant configuration, obtained updating, replacing, or integrating the Italian PV datasets of Ecoinvent database.

Adopting the Environmental Footprint impact assessment method, the environmental performances of the modelled Italian PV mixes (for 2021 and 2030) were evaluated and compared with those of the Italian PV mix of Ecoinvent database in function of 1 kWh of electricity produced.

The new PV mixes showed a significant reduction in environmental impacts, always greater than 40%, for all impact categories. This was mainly due to: (i) higher equivalent hours of operation; (ii) higher modules efficiency (iii) updating of the inventory data for the production processes of all PV plants components; (iv) addition of new datasets about the innovative PV technologies which are not present in the Ecoinvent datasets.

#### **5.02.P-Mo460 State of the Art, Challenges and Future Development of Environmental Assessments of Magnesia Supply Chain**

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The present study discusses the state-of-the-art environmental assessments of magnesia for refractory industry and highlights the key challenges to be faced for the development of a methodology able to support the progress towards sustainability. About ten studies were found calculating the Carbon Footprint or the Life Cycle Assessments (LCA) of dry-route processed magnesia. The lack of common approach and relevant inventories are considered the main weaknesses of the current situation. LCA scenarios for fused and sintered magnesia are proposed to support this conclusion and to fill some of the existing gaps. Two actions are suggested to improve the datasets: to include materials quality and to detail technical parameters. First, the quality of the materials along the supply chain plays a relevant role in the production design and in the functional value of the final product. As no complete tracking of the materials quality has been found in literature, its implementation in the LCA inventory is suggested as key future development. Besides, the expansion of the system boundaries towards the use phase is equally suggested, to directly connect the quality of the resources and the product functionality. This approach is expected to support the enhancement of the methodological challenge regarding resource depletion. Second, despite the well-known flowchart of magnesia production, many variations can occur in the choice of technologies and operating conditions, which result in different input/output flows for the product system, especially for energy consumption. A lack of transparency and completeness was encountered in literature, hence, a comparison of alternative production routes is not possible. The incomplete assessment of the existing routes makes the support to decision- and policy-making difficult and imprecise. LCA energy scenarios are proposed to show the dependence of the environmental burdens and the energy consumption. Concluding, the quality of dataset and inventory has been identified as limiting factor for the progress of the environmental assessment of the magnesia supply chain. An urgent effort is needed to improve data quality to gain a better understanding of the magnesia-related environmental burdens. The identification of the hotspots can orient the methodological research towards the most impacted environmental domains and those for which the largest variability is observed due to the low representativeness of the methodology.

#### **5.02.P-Mo461 Comparative Life Cycle Assessment of Power Supply Units in Direct and Alternating Current**

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Most of renewable energy sources (RES), electric loads and storage systems use Direct Current (DC), despite the electric grid is in Alternating Current (AC). For this reason, there are considerable conversions between AC and DC when RES are used. A photovoltaic panel, for example, produces electricity in DC that is converted to AC through an inverter; this electricity provides power to a computer that has inside a converter that turns AC to DC. This process causes at least two “useless”

conversions, with consequent energy and environmental impacts. In addition, the AC devices usually include more materials than DC ones, which can increase the above mentioned impacts.

This work aims to verify if the use of DC instead of AC devices can reduce the energy and environmental impacts (in particular Global Energy Requirement - GER and Global Warming Potential - GWP) from a life cycle perspective. For this purpose, a comparative Life Cycle Assessment (LCA) is conducted for a computer's Power Supply Unit (PSU) such as model of typical electric load that converts AC to DC. A PSU was disassembled at the University of Palermo's "LCA and Ecodesign Lab", all components of the PSU were catalogued and weighed, with a focus on the printed circuit board, to create a bill of materials model of the AC PSU. Starting from literature data, a model of DC PSU was created by identifying the components to be removed to transform a PSU from AC to DC.

Focusing on the two above models, as hypothesis, the steel case, the fan, and the cables are the same in both PSUs, while the printed wiring board of the DC PSU includes less materials and its surface is 25% less than the AC PSU. The eco-profiles of the two PSUs were calculated considering a "from cradle to gate" approach.

The results indicate that the DC PSU causes 186.3 MJ of GER, that is 38.5% less impact than the AC model, and releases 10.5 kg CO<sub>2eq</sub>, 37.4% less than AC PSU. The obtained results are valid for examined computer's PSU, but the approach can be extended to other electric loads to assess the energy and environmental advantages of using systems operating directly in DC.

Adopting DC technologies, integrated to RES and electronic loads, can be a key element for the ecological transition and can lead to the development of more sustainable and efficient DC microgrids with reduced environmental impacts.

The research is developed within the activities of the project LOV – Lowering Ortigia's Voltage, funded by PO FESR 2014/2020.

#### **5.02.P-Mo462 Solving LCI data gaps for fine chemicals by retrosynthesis for industrially relevant reactions**

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Life Cycle Assessment is a pivotal methodology for assessing and enhancing the environmental impact of products, processes, or services by analyzing material and energy flows within supply and value chains. Our study addresses a critical gap in the realm of Life Cycle Assessment (LCA) for chemical reactions, where challenges such as data scarcity, confidentiality concerns, and the quest for sustainable reaction routes hinder comprehensive environmental evaluations. These challenges are particularly pronounced in the fine chemical industry, exacerbated by the absence of crucial environmental emission factors and concerns about confidentiality. To overcome these hurdles, we introduced an automated approach that utilizes a three-step algorithm for retrosynthesizing relevant fine chemicals. By combining neural networks and forking the AIZynthfinder project, we tailored an algorithm to identify compounds within the industrial value chain. Our test set, comprising 5,551 'druglike' molecules, demonstrated the algorithm's efficacy in generating valuable LCA entries. The 182 solved retrosynthetic reaction routes need to be sanitized and used to generate forward reactions, which are then fed into the Carbon Minds database. This automated synthesis planning does not only addresses data scarcity but also accelerates the traditionally slower modeling approaches, providing a tool to streamline LCA for chemical reactions and facilitate informed decisions about sustainable and environmentally friendly production processes in the fine chemical industry.

#### **5.02.P-Mo463 How highly specific LCA data can inform purchasing decisions that significantly reduce environmental impacts**

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To address the growing need for environmental sustainability, many companies, particularly in the chemical industry, are exploring strategies to manage the significant costs associated with reducing their environmental impacts. Interestingly, only a small portion of emissions come directly from a company's own operations or from energy-related emissions from purchased energy supplies. The substantial part of environmental impacts originates from the upstream and downstream elements of their supply chains. The lack of detailed information on the environmental impacts of potential suppliers' production processes, however hinders companies to make informed decisions. To enhance decision-making processes, a new regionalized Life Cycle Inventory (LCI) database has been developed, adhering to ISO standards for Life Cycle Assessment (LCA), specifically ISO 14040/44/67 and TfS. This database focuses on the chemical and plastics sectors and features data on approximately 1,000 chemicals and plastics, covering up to 190 regions. In a case study the climate change impact of polypropylene production is analyzed, showing that the carbon footprint of the polypropylene supplier with the highest carbon footprint in a region is 11 times higher than the supplier with the lowest carbon footprint in the same region. As a results, these emissions can be reduced by up to 90% by choosing lower impact suppliers. This shows the importance of regionalized and detailed databases for informed decision making.

#### **5.02.P-Mo464 Advancing on the transition towards data interoperability: the Global LCA Data Access Network (GLAD)**

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The restricted accessibility of LCA data presents a notable obstacle to its widespread adoption, making it a pivotal concern within the LCA community. The harmonization of LCA data is being actively pursued by the Life Cycle Initiative of the UN Environment Programme (UNEP) through the Global Life Cycle Data Access Network (GLAD) initiative. GLAD is an open source web application, which allows users to find and access and screen metadata information to identify the most suitable datasets for use in LCA, ensuring interoperability by making connected datasets available in multiple formats, with compatible nomenclature and complete metadata. The development of GLAD has leveraged resources, expertise, and gathered requirements from diverse stakeholder groups from around the world, including the European Commission (EC). With most of the global data providers participating in GLAD, the initiative has been instrumental in supporting a worldwide endeavor to scrutinize interoperability challenges across LCA software, LCI databases, and LCIA methods. The GLAD Nomenclature Working Group (NWG) developed a procedure and a set of criteria to map elementary flows among major nomenclature systems and reviewed bidirectional mappings. The mapping procedure involved an ad hoc software tool titled the "GLAD Mapper Tool," which is made available by the EC. The tool is fed with the source and target flow lists and a file containing the mapping criteria properly formatted. The four nomenclature systems mapped within the NWG are ecoinvent, Environmental Footprint, IDEA, and the U.S. Federal LCA Commons. The Mapper Tool proved valuable for LCA practitioners, achieving over 90% coverage after seven iterations, with unique substances (flow names) and environmental compartments having the greatest impact. The NWG's mapping activities may serve as a starting point towards defining a central hub for mapping LCIA methods and datasets, improving data accessibility and interoperability for the LCA community as a step towards defining a unified nomenclature system. Relatedly and building on the mapping exercise, the Life Cycle Initiative, through GLAD, is currently working on developing the blueprint for a global nomenclature governance system (a central system) to map the elementary flows (EF) lists from major LCA databases. This approach holds the potential for greater interoperability across the LCA community, underpinned by GLAD's commitment to openness, inclusiveness and collaboration.

#### **5.02.P-Mo465 The global environmental footprint of metal commodities**

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The increasing demand for metal raw materials raises concerns as to the environmental burden of their production. This calls for a comprehensive, representative and reliable quantification of the environmental footprint of these raw materials in a life cycle perspective, based on up-to-date life cycle inventories (LCIs). However, current life cycle assessment (LCA) studies are often restricted to small-scale case studies that cover a relatively limited share of the global production. Thus far, only the study from Nuss and Eckelman 'Life Cycle Assessment of Metals: A Scientific Synthesis', published in 2014, provides LCA results for metal raw materials at a global scale. Yet, the latter study builds on market and LCI data prior to 2010, which is not representative of current mining/refining industry conditions. As a follow-up, this study aims at providing an up-to-date overview of the global environmental footprint of metal commodities, through the development of market-representative global LCA models for 62 different metal substances.

The development of these models follows a three-step approach based on:

- i) A market analysis, to identify the main commodities and production routes associated with each substance;
- ii) A review and analysis of existing LCI data in the literature and ecoinvent database, to select the most suitable LCI datasets in terms of market coverage, quality and representativeness;
- iii) LCA modelling, which combines the selected LCI datasets and market data to develop market-representative global models.

The approach is showcased using tungsten (W) as a case study. The LCI review and analysis eventually led to the selection of three LCI datasets covering three production routes: two primary routes relative to W mining and tungsten carbide (WC) production, respectively in China and North America; and one secondary route relative to ammonium paratungstate (APT)

recycling from hardmetal scrap. Based on these data, a first model was developed for the global primary production of WC, which is the main W commodity. The carbon footprint of the global primary WC production is calculated to amount to 73.3 kg CO<sub>2</sub>-eq/kg WC, driven in particular by the consumption of electricity and liquid nitrogen in the Chinese production route.

This preliminary W model needs further improvement. Moreover, beyond the W case, the approach is currently being replicated for the remaining 61 substances, for which updated LCI datasets will eventually be delivered.

#### **5.02.P-Mo466 PLIM-WIND: A Parameterized Life-Cycle Inventory Model to Assess the Impacts of the European Wind Turbine Fleet**

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The European Union aims to achieve an installed wind power of 510 GW by 2030, doubling the current capacity within eight years. This underscores the pivotal role of wind energy in European transition plans to sustainable energy sources. Currently, the assessment of the sustainability of wind energy deployment primarily relies on Life Cycle Assessment (LCA). However, a big challenge lies in the scarcity of updated open and accessible data concerning the technical aspects of wind turbines. For instance, widely-used LCA databases like Ecoinvent rely on wind technology data from 2001 to 2007. At the same time, recent inventories mainly cover 1-2.5 MW turbines, far from current market trends of installed power in Europe. This data mismatch highlights the challenge of improving the representativity of wind turbine life cycle inventories through modelling.

In this study, we present a novel parametric model, PLIM-WIND, designed to generate customized life-cycle inventories for wind turbines, encompassing both individual turbine and wind park scales and based on power and design. The comprehensive inventories span all life-cycle stages, from material extraction to end-of-life, incorporating not only the turbines but also other components such as cables, transformers and access roads. This model facilitates the integration of technological, temporal, and geographical parameters that influence wind park installation and electricity production, enabling a nuanced analysis. To prove the validity of the tool, we characterized the material requirements and land use of the European wind fleet up to 2021. In addition, we also computed the aggregated environmental impact indicators derived from Life Cycle Impact Assessment ReCiPe method and assessed the viability of the fleet. All in all, this model contributes with new inventories to better evaluate the sustainability of highly renewable electricity mixes like the one the European Union is aiming for.

#### **5.02.P-Mo467 Validation of New Technology for Sustainable Recovery of Materials in the Non-Ferrous Metallurgical Industry: RECOPPs Project**

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The RECOPPs project aims to validate a new technological solution, scaling it from a Technology Readiness Level (TRL) 5 to a TRL 7. The aim is for the developed processes to recovering of added-value raw materials Bismuth (Bi) and Antimony (Sb) as well as the removal and inertization of Arsenic (As) from residues and by-products generated during the pyrometallurgical production of Copper (Cu) in the industry.

The management of mining industry waste is a relevant issue in today's society. This is of high importance because, on the one hand, there are substantial quantities of critical raw materials that are currently essential for the development of new materials and technologies. Regarding this project, bismuth is crucial for use in the pharmaceutical and cosmetic industries, and antimony serves as a useful catalyst in the production of polyethylene terephthalate and flame retardants amongst other uses. On the other hand, toxic elements such as arsenic are also present, and its removal is essential to prevent its dispersion across various environmental compartments and to avoid potential adverse effects on ecosystems and human health.

Through life cycle analysis, the aim is to validate this new technology, identify potential areas for improvement, and demonstrate its viability considering both environmental and economic perspectives. This assessment will quantify the environmental impacts of the different routes developed for the recovery of Bi and Sb as well as the inertization of As, while assessing the environmental benefits provided by RECOPPs strategies. Additionally, aims to conduct a comparative assessment against the current situation of the management of waste streams from a pyrometallurgical industry located in Spain. To perform the LCA, the recovery of 1kg of Bi and Sb will be addressed as functional unit. Regarding the system boundaries, all the inputs and outputs of material and energy are shown, as well as the processes that conforms the treatment of the eluate. Furthermore, the specialised software SimaPro v9.1 will be used, along with the LCA database Ecoinvent v3.6 for the inventory of the system and the method chosen to calculate the environmental impacts will be ReCiPe 2016 Hierarchist v1.1.

This approach emphasizes the principles of the circular economy by reintroducing to the market secondary resources with significant added value generated within the industry.

#### **5.02.P-Mo468 The Grand Unifying Theory of Hybrid Life-Cycle Assessment**

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The reduction of truncation errors, the inadvertent omission of relevant emissions along the supply chain, remains an unsolved

problem in life-cycle assessment (LCA). Hybrid life-cycle assessment (HLCA) has long been proposed as a powerful solution to removing the truncation error and thereby improving the accuracy of (LCA) studies. In HLCA, data from economic input-output (IO) tables are combined with specific process-data from an inventory database. However, the complete hybridization of both the foreground and the background inventory would require a complete matching of processes to their corresponding economic sectors. While some authors have used a set of heuristics for matching, others have suggested the use of machine-learning (ML) algorithms for greater flexibility.

Despite the promises of HLCA, its application remains limited in scope. Even more importantly, the contested designation of different methods and the lack of a common notation means that the majority of case-studies claiming to use some form of HLCA cannot be reproduced. This is largely the result of wide-spread confusion concerning the implementation of HLCA.

Today, four distinct HLCA methods are recognized in literature: "Matrix Augmentation", "Integrated", "Tiered" and "Path-Exchange". While recent publications have attempted to compare these, the advantages and drawbacks of each method remain entirely obscure. What is more, in the "path-exchange method", where the use of ML was most recently suggested, the original authors in 2017 completely changed the underlying mathematical definition while claiming to adhere to their original 2009 publication.

In our work, we provide mathematical proof that three of the established methods are mathematically equivalent. We further show evidence that should strongly discourage the use of the "Path-Exchange" method due to the lack of connection between the process-based supply chain and the IO-system. We further extend the mathematical proof with a formal system of visual representations, allowing practitioners to quickly compare different methods. Finally, we lay out a pathway to the use of different ML algorithms for process matching in this new framework of HLCA, enabling the complete hybridization of LCA databases.

This work represents an important step towards the wide-spread use of automated HLCA for higher-accuracy LCA results, which will become more relevant as further environmental footprinting policy is implemented around the world.

### **5.03.A LCA and Beyond – Integrating Sustainability and/or Other Dimensions for a More Informed Decision-Making**

#### **5.03.A.T-01 Methodology for the Life Cycle Assessment of Various Process Design Options in Alternative Fuel Production**

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Sustainable transport fuels provide an essential alternative to fossil fuels when aiming to reduce greenhouse gas emissions in the EU transport sector, especially for the transport that is difficult to electrify. The production can be achieved via multiple process routes and even different process designs within one route. Hence, the question arises how to easily and comparably assess such a variety of process designs from a techno-economic and ecological perspective.

Within DLR the in-house tool TEPET<sup>+</sup> was developed to perform semi-automated techno-economic analyses (TEA) as well as life cycle assessments (LCA) of chemical processes and technologies. Both TEA and LCA build upon a rigorous energy and mass balance of an Aspen Plus® simulation. By combining TEA and LCA in one tool a consistent assessment (same boundary conditions, functional unit etc.) of different process designs is enabled and economic and ecological trade-offs can be quantified. The application of the tool and methodology regarding the LCA and combined evaluation is demonstrated for a Biomass-to-Liquid process with two biomass sources (bark and straw) and three process configurations.

While there are slight differences in environmental impact between the two biomass types, the variances between configurations are more prominent. In dependence of the major impact contributors the configuration without external electricity demand or the one with a lower biomass input to product output ration has the lowest impact. The combination of economics and ecology in the greenhouse gas abatement cost reveals a trade-off that favours the configuration with the lowest cost but medium global warming potential.

Semi-automated LCAs enable the easy and comparable assessment of multiple process designs while the combination of LCA and TEA in one tool allows for a consistent sustainability assessment from both an economic an ecological point of view. Although this methodology was only illustrated for one sustainable fuel production process, it can be applied for various other chemical processes as well.

#### **5.03.A.T-02 Exploring the integration of Risk Assessment and Life Cycle Assessment in the context of the Safe and Sustainable by Design framework**

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Approaches to safety and environmental impact assessment are pivotal for protecting environment and human health and ultimately for sustainability assessment. However, over time, the development of the approaches to safety and sustainability has been mostly independent leading to different fields of expertise, e.g. risk assessment (RA) and life cycle assessment (LCA). The evolution of decision making needs for sustainable chemicals, materials and products is now requiring the integration of safety and sustainability considerations, supporting ex ante assessments, capitalizing on the knowledge generated by the different approaches, including the identification of trade-offs and the evaluation of impacts at different scale (from local to global). The integration of RA and LCA has been widely discussed in literature, but the complete operationalisation of the approaches in tandem is challenging.

The Safe and Sustainable by Design (SSbD) framework for chemicals and materials as recommended by the European Commission pushes for an integration of the two approaches. The ultimate goal of the SSbD framework is to promote innovation towards safer and more sustainable chemicals/materials. It can be applied in the chemical design phase or to compare existing chemicals. Integration of RA and LCA aims at addressing the assessment needs streamlining the practical steps. Among the possible integration options identified by previous studies (e.g. results combination, RA into LCA, LCA into RA, etc.), the core challenge is to identify the one that is most suitable in the context of the SSbD framework..

The present study explores the extent to which safety and environmental sustainability assessment should be integrated. To this end, we firstly understood the terminologies used under the umbrella of this integration. Secondly, we performed in parallel RA and LCA following the steps of the SSbD assessment and we detected a number of preliminary data and assumptions to be harmonized, for instance the ones already embedded in the methods and models used for the assessment. The introduction of a preliminary step that clearly defines the study goal, here proposed, would improve the SSbD assessment by collecting information that will be used by both assessments, especially when different assessors perform the two assessments in parallel.

### **5.03.A.T-03 Human toxicity assessment in LCA and as element in Portfolio Sustainability Assessment**

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Although regulatory requirements are in place to ensure the safe use of chemicals in their application, the ambition to reduce the environmental impacts of products along their life cycle is often combined with an ambition to identify and reduce hazards and exposures. As there is currently no methodology available to compare hazard and exposure performance along the lifecycle and identify priorities for improvement, requirements are often based on purely hazard-based lists for priority substances without evaluating their risk in specific applications. The new TripleS approach (Sustainable Solution Steering) was published in 2023.

The new method contains updates reflecting simplifications in the segmentation process, increasing transparency of the process and allowing for automation. Furthermore, changes in chemicals legislation have been reflected, ensuring the functionality as an early warning system for portfolio management. The purpose of this manual is to document the process for BASF's TripleS approach. The manual aims to describe the segmentation methodology and relevant processes, including roles and responsibilities of participants involved. The TripleS process, as presented hereafter, fulfills the following quality criteria, based on the International Standard on Assurance Engagement (ISAE) 3000.

As an additional element of Triple S LCA elements can be integrated to quantify the overall environmental impact of products along the life cycle but the models for toxicity impacts are focussing on the indirect impact of chemicals emitted into the environment. As a performance-based indicator for the application in LCA; ProScale assesses hazard and direct exposure potentials from chemicals along their life cycle. It can be integrated in LCA to compare human toxicity potentials of alternatives.

The presentation shows elements of the TripleS method and how it helps to meet the needs of customers, value chains, governments and society. TripleS was introduced at BASF with the aim of increasing our portfolio of innovative and sustainable solutions and the sustainability performance of the value chains we serve. By assessing key drivers and issues in our customers' industries, we strive to identify the sustainability contribution of each of our products in its specific application. To do so, solutions in their respective application and region are assessed in terms of defined sustainability criteria evaluating the value chain from cradle to grave.

### **5.03.A.T-04 Integrating nutritional aspects and planetary boundaries in food LCA**

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Intentions to include nutritional considerations in life cycle assessment (LCA) of foods have increased. The main way to do so has been using nutritional functional units (nFU), and nutrient indices composed of several nutrients have proposed to be utilised. However, nutrient indices are initially intended for nutrition education to promote healthy diets and therefore are not necessarily the most suitable as nFUs directly in the comparisons of individual products in the context of LCA. Key questions are, should the different product groups be addressed more specifically, what product categorisation would be suitable and how

the nutrients of each nutrient index should be selected. The aim of our study was to further develop a product group specific approach to include nutritional aspects in FU in several product groups and progressing to a methodological framework for defining sustainable food products. We developed nFUs to protein sources, carbohydrate sources, vegetables, fruits and berries group, fats, and meal drinks. We evaluated different strategies to select the nutrients to be included in the nutrient indices and conducted a validation study to evaluate the suitability of the strategies for the baseline use that refers to the current dietary situation. However, the selection of nutrients for the index is always closely related to the dietary context in which the results are to be utilised, so we applied the approach in two different dietary contexts, the Finnish and the Spanish. In addition, we developed a new method for combining the nutritional basis with planetary boundaries-based life cycle assessment (PB-LCA), and we applied it in the Finnish case. As a result of the study, there are two sets of product group specific nFUs that have been adapted to the diet that reflects the local food culture. Use of these nFUs brings important sustainability information to product selection situations. The study provides a sophisticated and compelling way for the downscaling of planetary boundaries to food products considering the different roles different foods play in providing nutrients. According to our results, a large part of the products will be defined as unsustainable. This challenges the food system and diets, but also raises the question of whether it is possible to reach the share of the planetary boundaries assigned to the food system or whether the share of food should be increased in the method of downscaling the planetary boundaries.

### **5.03.B LCA and Beyond – Integrating Sustainability and/or Other Dimensions for a More Informed Decision-Making**

#### **5.03.B.T-01 Evaluating biofuel alternatives for passenger transportation through sustainability indicators**

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Liquid biofuels are pivotal in driving the transition to a sustainable transportation sector, providing a pathway to decrease carbon emissions and seamlessly integrate with the current vehicle fleet and infrastructure.

Biofuels can be produced using different sources and processes, each of which generates different environmental impacts and achieves different engine performances. In this context, the identification of the most suitable biofuel production routes is a valuable piece of information so that researchers, policymakers, and companies can explore and promote these options.

This study explores 19 biofuel production routes as potential substitutes for diesel in passenger transportation, encompassing seven bio-oil feedstocks and three distinct biofuel production processes. We characterize the performance of these routes utilizing key indicators such as cost, water use, land use, and global warming potential, so as to comprehensively cover the three dimensions of sustainability: economic, environmental (based on life cycle assessment), and social. With these data at hand, we employ a multi-criteria decision making tool to systematically evaluate biofuel alternatives and rank them to identify the most promising options and draw general conclusions.

Notably, biofuels derived from waste, such as tallow, emerge as standout alternatives, boasting lower environmental impacts compared to others. Conversely, emerging technologies like microalgae exhibit less favourable performance. This research serves as a pivotal resource, offering valuable insights to shape policies and initiatives aimed at enhancing the sustainability of biofuel alternatives with improvement potential.

#### **5.03.B.T-02 Is Corporate Growth Sabotaging Sustainability Goals? A Case Study of Photochromic Textiles**

**Kamal A. Kamali, Bertrand Laratte and Guido Sonnemann, University of Bordeaux, France**  
Constant revenue growth is a key indicator of corporations' success. As corporations adopt various growth strategies relying on innovation to expand their market share, they may inadvertently trigger an increase in environmental and social issues. For example, single use coffee capsules, foldable smartphones, and wireless chargers can be attributed to higher waste generation, shorter lifetime causing frequent replacements, or higher energy consumption, respectively. Moreover, the demand for high quality yet affordable products often drives companies to shift their production to developing countries. This decision is driven by the potential cost reduction associated with lower wages, reduced social security expenditures, and lower standards for health and safety of the workers, thus resulting in high social risk.

Climate change various detrimental impacts on the environment, economy, and human health led world governments to sign the Paris Agreement. However, we are currently off course and to limit global warming to below 2°C, a 30% reduction in greenhouse gas emissions is necessary by 2030. This necessitates conducting sustainability assessment on emerging technologies to ensure their environmental and social sustainability, ensuring that prioritizing reduced greenhouse gas emissions technologies does not worsen social well-being of different stakeholders.

The fashion industry known for its substantial market size and high compound annual growth rate, serves as a compelling case study illustrating how certain growth strategies can affect consumption habits, resulting in accelerating climate change, increasing social risks, and hindering progress towards the sustainable development goals. Therefore, a prospective

environmental and social analysis of photochromic textiles, a type of fabric that changes color when subjected to UV light, is conducted.

This work investigates how the adoption of innovation driven business models prioritizing constant revenue growth can influence workers' well-being and the progress towards attaining the Paris Agreement targets. We aim to uncover the global warming potential and the potential social impacts of these textiles in 2030 when technological maturity has been reached. As a result of this work, it was evident that incorporating photochromic dyes in fabricating clothing fabric does indeed offset a part of the progress attained by shifting towards renewable energy production and cause higher potential social impacts.

### **5.03.B.T-03 Societal Life Cycle Costing for the definition of eco-design requirements of products: a methodological proposal**

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Societal Life Cycle Costing (sLCC) assesses all costs associated with the life cycle of a product that are borne by anyone in society. It includes all external costs, whether soon to be internalised or not, expressed in monetary terms. For this reason, sLCC is especially relevant for public authorities interested in knowing the overall cost of a product, through the monetization of societal and environmental impacts, as well as the computation of indirect costs held by actors not directly involved within the product life cycle (Bianchi et al., 2021).

This contribution aims to present how sLCC is applied in the context of eco-design policies developed by the European Commission (EC). In particular, sLCC is part of a framework, which has the objective to set and prioritise eco-design requirements for specific product groups to improve their circularity, resource and energy performance and other environmental sustainability aspects. sLCC is combined with an analysis of environmental impacts, performed through a Life Cycle Assessment (LCA), and with a qualitative assessment of social impact and non-quantifiable aspects and policy objectives.

First, monetisation factors are discussed, focusing on those available for the monetary valuation of midpoint impacts according to the 16 impact categories assessed by the Environmental Footprint method (EC, 2013a, b and related updates). The factors are used in the weighting phase of LCA. Secondly, the procedure followed to subtract environmental taxes and subsidies is presented, so that these transfer payments are not included, as they do not lead to a net cost effect for society as a whole. Finally, future methodological developments are proposed.

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### **5.03.B.T-04 Sustainability Assessment Framework for Innovative Technologies in Agriculture - Application to Algae Production as Alternative Feedstuff**

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Calls for technological innovations that contribute to sufficient, healthy and sustainable food are increasing. Comprehensively assessing the sustainability of novel agri-food technologies is however challenging because of their evolving character, uncertain social uptake, trade-offs between sustainability dimensions and impacts, the great variety of stakeholders affected, and the range of existing sustainability assessment methods. We propose a Life Cycle Sustainability Assessment Framework (LCSA) aimed at assessing the sustainability of novel agri-food technologies by providing guidance on choosing methods, ensuring stakeholder involvement, and linking social acceptability to social desirability as required by Responsible Research and Innovation (RRI). The proposed LCSA framework follows the four stages of life cycle assessment (LCA), considers the entire life cycle of the technology, and ensures multi-criteria evaluation. Stakeholders are involved in goal and scope definition, the description of the technical and reference system, the choice of impact categories, and data collection for the foreground system. Newly compiled lists of advantages and disadvantages of social and economic assessment method inform the choice of respective methods. LCA evaluates the environmental dimension. Stakeholder involvement is a central element of the method because literature supports its direct link to the social desirability aimed for in RRI. The pursuit of social desirability implies that stakeholder involvement in LCSA can contribute to the social acceptability of the technology. The developed LCSA framework guides an evaluation of the sustainability protein production for feedstuff from microalgae based on three vertical tubular photobioreactor systems (glass, silicone or polymethylmetacrylate). This case study illustrates the applicability of the developed LCSA framework. The comparison is at laboratory scale and the algae's suitability as input in a

livestock diet is assumed, but not further investigated. The LCSA framework is usefully applicable to the production of microalgae as alternative feedstuff, while thorough stakeholder inclusion requires large resources and the comparison with the reference system requires substantial calculations.

### 5.03.P LCA and Beyond – Integrating Sustainability and/or Other Dimensions for a More Informed Decision-Making

#### 5.03.P-We400 Exploring the Environmental and Economic Sustainability of a Novel Light-Driven System for Wastewater Management

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Among current CO<sub>2</sub> valorisation technologies, one promising approach is artificial photosynthesis for energy production/storage. However, technological development and robust analyses are required to ensure the sustainability of light-driven systems. This work presents a multi-dimensional analysis, combining ex-ante environmental life cycle assessment and life cycle costing methodologies, of a novel photoreactor system for simultaneous treatment of water pollutants and fuel production from sunlight. Given the exploratory nature of this work, two different functional cases were considered: (i) only wastewater treatment, and (ii) wastewater treatment combined with solar fuel production. System boundaries of the photoreactor system include the manufacturing of the cell, stack and prototype, as well as its operation. The life cycle inventory was obtained through direct communication supplemented by life-cycle and economic databases. The Environmental Footprint method was used for the life cycle impact assessment, considering municipal wastewater treatment (in the first functional case) and the production of fuels such as ethanol, methanol or ethylene (in the second functional case) as reference systems. Scenario analysis was conducted to assess different operational scales of the system varying the number of cells, the prototype's lifetime, and electricity consumption. In order to estimate the levelised cost, a bottom-up cost analysis approach was followed by considering capital, building, operational, material and labour costs of the system. Although the carbon footprint impacts of operating the photoreactor surpass those of their conventional counterparts in both cases, potential environmental viability could be achieved provided that the system is multifunctional (producing fuel at a rate > 100 g/h) and scaled up. Regarding the photoreactor fabrication cost, the cells would account for the highest contribution. Comparing the calculated levelised cost of wastewater treatment with the cost for a conventional wastewater treatment system, simultaneous fuel production (producing fuel at a rate > 200 g/h) would be necessary to make this system cost-competitive. Hence, the conclusions drawn from this study emphasise two key prerequisites for the future environmental and economic viability of such a novel photoreactor system: (i) it should be multifunctional, and (ii) scale-up in design and operation is vital.

#### 5.03.P-We401 Evaluating Economic Sustainability in LCSA – Learnings from the International Round Table of Materials Criticality

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Raw material criticality is often suggested as an aspect to consider in an economic sustainability assessment in the Life Cycle Sustainability Assessment (LCSA) framework. In parallel, the project IRTC (The International Round Table on Materials Criticality) has addressed the questions of how criticality can be best evaluated and mitigated – although not in the context of LCSA. This contribution aims to identify best practices identified in the IRTC project that merit an integration into current LCSA practices.

Within the IRTC project, a cause-and-effect diagram is established and implemented in a free web-based tool that links raw material data, supply-risk factors, a company's exposure to these three types of risk, and the effect of these risks, to economic damage, both at a company and societal level. Recent studies discussing the integration of raw material criticality into the LCSA framework are reviewed to identify to what extent the IRTC approach is aligned with the current LCSA framework, and whether mutual improvements could be considered.

In the comparison between the IRTC approach and the supply risk methods currently recommended for the LCSA framework, many parallels and distinctions can be observed. A focus is put here on one difference: the IRTC emphasizes that raw material criticality is stakeholder dependent: economic value is created throughout the value chain, and each value-chain actor may operate in a different regional economy, and have different options for substitution (at the raw material, component, or technology level). On the contrary, LCSA methods do not seem to give room for multiple stakeholder perspectives. The lack of identification of a specific economic stakeholder in LCSA-focused criticality approaches diminishes the value of the assessment for all economic stakeholders. To avoid this, we suggest applying a step-wise procedure in economic sustainability assessments, allowing for the identification of sources of potential economic instability and potential mitigation measures. An economic sustainability assessment following this approach is less quantified than an approach based on characterisation factors that calculates a final single score. However, it emphasizes the interplay between supply disruptions, life cycle costs, and mitigation measures, and applies a defined stakeholder perspective, allowing for the provision of information that can lead to concrete actions.

### 5.03.P-We402 Evaluating the Sustainability Level of Energy Storage Options

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The intensified focus on environmental sustainability necessitates a shift to cleaner, intermittent energy sources like solar and wind, challenging the stability of electrical grids. Energy storage is a solution to match the intermittency of renewables with fluctuating energy demand.

This contribution aims for evaluation of 25 energy storage options. First, we categorize the options into medium-term (nine technologies) and long-term (16 alternatives) choices, to ensure a fair comparison. Indicators like energy consumption, energy density, employment (indicating new job creation), global warming potential, and water usage, resulting from Life Cycle Assessment (LCA) studies, assess the performance of each option. The application of Data Envelopment Analysis (DEA), a linear programming method, allows to integrate these sustainability dimensions into a single efficiency score. Alternatives with an efficiency score of 1 are deemed efficient, while those with scores strictly below are considered inefficient. In addition, DEA provides quantitative improvement targets for inefficient alternatives that, if achieved, would make them efficient. This is a valuable piece of information to guide the efforts of technology developers.

We find that, in the medium term, nickel-cadmium, Li-ion, sodium-sulphide, and lithium-ferro-phosphate batteries show relatively higher efficiencies. Conversely, vanadium redox flow battery, zinc bromine flow battery, lead-acid, and sodium-nickel-chloride batteries exhibit lower efficiencies, necessitating further improvements. Improvement targets reveal that inefficient batteries need over 80% reduction in water usage and global warming potential, demanding intense research and development efforts.

Among long-term options, green ammonia from solar consistently demonstrates high efficiency, followed by green hydrogen from solar and wind, and green ammonia from hydropower and wind. Grey (fossil fuel-based) and blue (fossil fuel-based with carbon capturing) options exhibit relatively lower efficiencies due to elevated global warming potential and energy consumption. Notably, grey hydrogen alternatives, like coal gasification, water splitting, and steam methane reforming, have substantial improvement targets (over 80%) in energy consumption, water usage, and global warming potential. In contrast, green options need relatively lower improvements.

The study offers insights for policymakers, energy planners, researchers, and technology developers.

### 5.03.P-We403 Second Life of Batteries: Modeling Choices in Social Life Cycle Assessment

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The increasing market of batteries and the need for a more circular economy entails the necessity to assess the potential of second-life batteries. Social life cycle assessment (sLCA) is not widely applied to batteries and only a few studies include their end of life, but without second life. This study aims to assess the social impacts of three batteries (from generation 2 to generation 4) and focuses on the methodology to include alternatives for a more circular battery over their entire life cycle.

This study is a type 1 sLCA using a reference scale approach and PSILCA. The system boundaries follow a cradle-to-grave perspective, comprising battery manufacturing from raw material extraction, use stage in Belgium, and end of life in Belgium. Second life is assumed to be in a domestic 4 kWp photovoltaic installation. The main modeling choice studied here is the functional unit (FU). FU1 is 1 kWh of electric energy delivered by the battery over its first life and credits from the avoided production of a new battery are included. FU2 is 1 kWh of electric energy delivered by the battery over its full lifetime (first and second life). Primary data for the batteries and their use stages was collected. Battery cells were tested under driving cycles. The costs of battery manufacturing are adapted from BatPac version 5.0. The battery manufacturing country is China and the component and active material are assumed to come from to the main respective producing countries.

The results show that in all cases, China is the main affected country, followed by Belgium. For FU1, the more advanced battery has lower impacts, and accounting for the avoided battery reduces the risks however as generation 3 also has less qualified batteries for second life, the credits from second life are lower. On the other hand, generation 2 with lithium titanate oxide as anode active material is a very long-lasting battery, explaining the high credits from second life. For FU2, almost the same conclusions can be drawn but the absolute values are up to twice lower than for FU1.

The modeling choices presented here allow us to conclude that second life reduces the social impacts of batteries. However, modeling an avoided battery production may not be recommended as it entails uncertainty around the choice of avoided battery and raises ethics concerns. In further steps, other modeling choices, such as allocations will be studied to confirm the potential benefits of circularity for batteries.



### 5.03.P-We404 Prospective life cycle assessment of organic redox flow batteries

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Organic redox flow batteries are emerging as a promising stationary energy storage solutions due to their versatility in meeting power and energy requirements across various application scales, as well as their long lifespan, low self-discharge, heightened safety, and the avoidance of expensive minerals. Evaluating the environmental performance of technologies during their early development stages is crucial for identifying potential environmental impacts and guiding technology development. This study assessed the environmental performance of two emerging TEMPO-based redox flow batteries: an all-organic flow battery (OFB) and a hybrid flow battery (HFB), using life cycle assessment (LCA). The environmental impacts of the two batteries are benchmarked to the conventional vanadium flow battery (VFB). Two functional unit were considered: 1 kWh theoretical storage capacity and 1 kWh electricity delivered over the battery lifetime. This study constructed a battery design model based on industrial data and design equations, calculating the required amount of battery materials. Additionally, a battery performance model was built to estimate the amount of electrolyte required and the energy delivered over the battery's lifetime. Moreover, a global sensitivity analysis (GSA) was performed to discover the relative contribution of input parameters to the total uncertainty. Overall, the results showed that OFB and HFB outperformed VFB in most impact categories, except for freshwater ecotoxicity and resource depletion. Regarding the relative contribution of battery components, the energy subsystem are the biggest contributor to the total impacts regardless of the battery type, functional unit, and the environmental impact categories. This is associated to the high environmental emissions of the electrolyte. GSA highlighted that electrolyte capacity fade is the parameter affecting the results most, emphasizing the need for battery researchers to prioritize improvements in this performance parameter for the development of sustainable flow batteries.

### 5.03.P-We405 Combining Life Cycle Assessment and Planetary Boundaries for Sustainable Energy System Designs

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The global energy system is responsible for over 73% of global greenhouse gas emissions and is, thereby, the main driver of climate change. Hence, to mitigate climate change, energy systems must shift towards net-zero greenhouse gas emissions. However, the shift towards net-zero may be accompanied by a shift in the environmental burden of energy systems from climate change towards other environmental impacts, possibly compromising the absolute environmental sustainability of energy systems. Life Cycle Assessment (LCA) enables the identification and quantification of burden-shifting, but does not facilitate the assessment of absolute environmental sustainability. By integrating the planetary boundary framework into LCA, absolute environmental sustainability assessment of energy systems becomes feasible. Current absolute environmental sustainability assessments of energy systems solely focus on single-sector energy systems without analysing cross-sectorial interactions and fail to identify obstacles and enablers of absolute sustainable energy system designs. We employ an energy system modelling and optimisation framework with integrated LCA (SecMOD) for the case of Germany that encompasses multiple, coupled energy sectors, to assess energy system designs in terms of absolute sustainability, identify critical technologies preventing absolute sustainability of energy systems, and utilize multi-criteria optimization methods to span a design space for absolute sustainable energy systems. We find that an absolute sustainable energy system for Germany cannot be achieved by an autarkic and sector-coupled energy system if energy demand is not reduced. We disclose the main technologies in the German energy system's supply chain compromising absolute sustainability. To enable an absolute sustainable energy systems, we span a design space including energy imports and energy demand reduction, e.g. by increase of energy efficiency. For the obtained energy system designs, we also provide cost estimates. We emphasise the need for considering environmental impacts beyond climate change in the design of future energy systems, as environmental burden-shifting prevents the absolute environmental sustainability of energy systems. Additionally, we conclude that absolute sustainable energy system design can be achieved through energy demand reduction and energy imports.

### 5.03.P-We406 Life cycle assessment of future energy system flexibility – a methodological framework applied to Belgium

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Future European energy systems are built based on a higher share of renewable energy technologies, such as wind and solar, requiring storage to match energy supply and demand. Simultaneously, transport decarbonization is expected to increase the share of electric vehicles (EV), which are currently only used around 5% of their total time. Hence, the remaining 95% of their time, the EV could be used to help to balance the electricity grid, known as vehicle-to-grid (V2G) technology. To reveal its environmental benefits, a framework is needed to understand first the provided electricity by V2G service for the grid at each given hour and to translate this into environmental impacts. Currently, no such metric exists for evaluation of environmental impacts of V2G service in a future energy system.

Thus, this paper proposes a framework to assess the environmental impact of V2G services by combining prospective life cycle assessment (PLCA) with an energy system model. In this study, the functional unit is selected to be one kilowatt-hour generated by the Belgium electricity market in 2050. System boundaries include a cradle-to-gate approach for all generating

electricity assets. Additionally, prospective life cycle inventory databases are built for three Belgium energy scenarios in 2050. To obtain the hourly electricity supply mix, an internal developed design- and optimization framework, an optimal economic dispatch model used for small and medium energy systems, is upscaled to a national level. Furthermore, the Belgium EV fleet is simulated to supply V2G service. In a last step, the output of the hourly national dispatch model is reduced by the electricity supplied with V2G, the environmental impact of the new supply mix is calculated and compared to the impact of the current electricity mix.

The proposed framework is an example of integrating PLCA with an energy system model to evaluate the environmental impacts of an emerging technology. Results are presented at the SETAC meeting itself.

The proposed methodological framework and its application to Belgium can support policymakers to advocate for a broader application of V2G technology and faster adoption of bidirectional chargers and thus use the unused storage capacity of a growing EV fleet to integrate more renewable energy into the electricity grid. This framework can also be adjusted for other countries and thus be relevant for other researchers and policymakers outside of Belgium as well.

### **5.03.P-We407 Social Implications of Raw Material Requirements for Solar Photovoltaic Deployment on the Terawatt Scale**

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The urgent need to meet climate goals requires a massive expansion of renewable energy technologies, e.g., solar photovoltaics (PV). However, the large-scale deployment of solar PV presents challenges intricately linked to the availability of raw materials and its environmental and social implications. While previous studies have focused on resource constraints and environmental implications, social concerns remain largely unexplored. This study aims to fill this gap by assessing the social risks associated with the prospective material requirements for solar PV deployment. Initially, the prospects for solar PV in terms of global installed capacity were determined using the Integrated Model to Assess the Global Environment (IMAGE) for three specific years: 2030, 2050, and 2100. The study assumes that the solar PV technology mix will consist of 90% silicon (c-Si), 5% cadmium telluride (CdTe), and 5% copper indium gallium selenide (CIGS) solar PV. Eight raw materials were selected, and their material intensity per installed capacity was estimated based on a literature review. Two scenarios were defined to represent the minimum (best-case scenario) and maximum (worst-case scenario) demand for raw materials. Regarding the estimation of the social profile related to those materials, the study assumes that PV production is concentrated in East Asia and Southeast Asia. For these regions, two types of data were collected: i) the identification of the mining countries supplying materials to these regions based on the COMTRADE and USGS databases, and ii) the number of worker hours associated with mining. This information was integrated into the PSILCA database to ultimately derive the material-related social profile for the target years. The results for four selected indicators (child labour, frequency of forced labour, fair salary, and health expenditure) reveal that the large-scale implementation of PV capacity worldwide is directly associated with an exponential increase in potential social impacts in countries where raw material mining occurs. Efforts to improve efficiency in material intensity could reduce these social impacts by 9-16%. However, considering the nature of these impacts, it is crucial to aim for zero-risk targets in the medium-to-long term, monitoring and ensuring the respect of human and working conditions as a vital strategy within energy planning.

### **5.03.P-We408 Life Cycle Assessment of Emerging Hybrid Storage Systems for Maritime Sector**

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The electrification of transport has been emerging as one of the key strategies towards the transport decarbonisation pathways since it offers the possibility of replacing fossil fuels used in internal combustion engine with renewable energy sources.

Battery energy storage systems (BESSs) are the dominating storage technology for electric transport. However, heavy duty-cycles and high dynamic response typical of the maritime transport limit the employment of this technologies since oversized BESS are necessary. In this context, the adoption of hybrid storage systems (HSSs), combining BESS with either supercapacitors (SCAs), superconductive magnetic energy storage systems (SMEs), or both, is explored within the EU-funded V-ACCESS project to enhance the readiness level of HSSs and boost their implementation on electric propelled vessels.

At this early stage of technology development, Life Cycle Assessment (LCA) is necessary to orient research towards the better compromise between technical/design requirements and environmental sustainability.

In this context, within the V-ACCESS project, the authors aim at investigating the environmental system-wide trade-off of HSSs against BESS in three types of vessels. In detail, the examined systems consist of (1) full electric ferry, (2) hybrid offshore supply vessel, (3) hybrid trawler. The analysis will be carried out through the LCA methodology. The selected functional unit is “1 kWh of the total electrical energy provided over the service life by the power system technology to perform a specific function”. The analysis will follow a “cradle-to-grave” approach. For each case study, detailed primary data (foreground processes) about SCAs and SMEs manufacturing processes and vessels operational profiles will be provided by

the industrial partners involved in the V-ACCESS project, while the end-of-life treatments will be modelled based on the practice available at the commercial scale. The background processes will be modelled based on the Ecoinvent 3 database.

The study will allow to compare the implementation of HSSs against BESS based on a wide set of environmental data in accordance with the sustainability objective of avoiding burden-shifting among impact categories. Moreover, it will identify the “hot spots” and provide a useful support to industries towards the eco-design of the examined system. The obtained results can support decision-making processes towards the transport decarbonization pathway.

### **5.03.P-We409 Relevance of application and use-phase impact on Carbon footprint of Battery Energy Storage Systems (BESS)**

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One of the most mentioned aspects of battery installations today is their (often large) carbon footprint. Reported values mainly cover production phase and estimates for End-of-Life (EoL) associated impacts of Battery Energy Storage Systems (BESS). While studies show the significance of use-phase emissions in the BESS life-cycle, applications vary greatly and system boundaries (geographic and temporal) are critical for proper quantification. Hence, reflecting these differences and providing a complete value that can be comparable between systems is a challenge. The purpose of this work is to show preliminary quantitative characterisation of the use-phase associated impact and the differences that different applications can have within the same or at least similar local energy system boundaries. This was produced using different application specific models developed in Python and Excel. The models were developed to reflect capacity services (such as energy arbitrage, capacity firming) and ancillary services (frequency regulation). These models indicated the characteristics of BESS operation under different scenario constraints, as well as compared those to the StatusQuo reported carbon footprint values for similar systems. Furthermore, different location-specific rules and policy constraints are investigated and simulated to understand how such system behave in the real world in those location- & use-specific scenarios. Reported values by the models show that the use-phase associated emissions can significantly surpass the production and EoL phase values. Additionally, levelising the impacts across the system lifetime, a more accurate understanding can be produced to benefit different stakeholders, from industrial actors to policy makers. Findings show that understanding the location, time and service-specific is critical for adequate assessment of associated carbon emissions to a specific system. The term application-complete carbon footprint was coined to represent the final value that includes the use of system, and application-incomplete carbon footprint represents the previously reported values. However, both delineations can be shown to serve an important role in different contexts; from project/system design, optimisation, and decision-support; to limiting and/or allocating responsibility across the value chain of BESS. This produced understanding and implications can serve an important role in policy and community/infrastructure planning as well.

### **5.03.P-We410 Comparative life cycle assessment of NMC 811 type Li-ion battery production using different electricity mix**

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The need for battery storage device is rising and with this, the demand for battery production is on the rise too. Previous studies on the environmental impact of lithium ion batteries (LIBs) revealed that cell production within overall value consolidates the most energy-intensive stage. In addition, the energy usage during this process contributes heavily to the environmental impacts too.

In this study, the energy consumption and GHG emissions related to the production of NMC 811 battery cell will be evaluated. Different European electricity mix will be considered for this study- the Czech Republic, Hungary, Poland and Slovakia. According to Bloomberg News Energy Finance (BNEF) 2022 ranking, these four nations featured in top 30 countries, globally, for contributing to the battery supply chain. In the report, China accounted for 77% of global battery manufacturing capacity and Europe stands at 14% of the global share.

For comparison purpose, a Norway electricity mix will be taken into consideration. It should be noted that the electricity mix generation of these countries are mostly reliant on non-renewable sources, whereas, about 98% of electricity mix generation in Norway comes from a renewable source. These countries, with variance in sources of electricity mix production will be analysed, evaluated and compared using life cycle assessment method. Thus, evaluating the environmental impacts from the NMC 811 cell production in its geographical context.

The regional variability analysis of the cell production step studied in this work will set the platform on the need to harness renewable energy for the generation of electricity which, in turn, will make battery production more sustainable. Additionally, the future directions will be identified for achieving carbon-neutrality in line with the European Union (EU) battery regulations.

### **5.03.P-We411 Life-Cycle and environmental impact assessment approach to support decision-making in hybrid Solar-Battery projects**

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Energy projects today are designed (and optimised) for economic constraints and objectives, such as minimising cost and maximising profit/short payback period. With sustainability targets, environmental constraints are becoming increasingly relevant to take in consideration. This poses a challenge of satisfying potentially competing objectives, where the a more profitable energy system could perform worse on environmental indicators than a less profitable alternative. With it comes an opportunity to apply the understanding of Life-Cycle Thinking and Life-Cycle Management approach in the methodological framework. With this addition, a more relevant context and support for decision making and energy project design can be achieved. This approach can also leverage Life-Cycle Assessment as a supporting methodology in early stages of project implementation and development, as it is usually used as a forensic tool after the project decision has already been made. For this purpose, a model representation of a Solar-BESS system was developed to assess and optimise system performance and design in above mentioned competing objectives. The identified trade-offs show the discrepancy between economics-oriented and environmental-oriented solutions. Extending to different scenarios and conditions, the produced information can support different stakeholders in the decision making process for different objectives/goals. Furthermore, it was showed how the produced information and insights vary depending on location-specific conditions. Reported values show that the use-phase associated emissions can significantly surpass the production and EoL phase values, even when levelised and accounting for necessary system replacement in the project lifetime. Furthermore, trade-offs in optimisation approach have been identified in the context of projected global (local) energy system development when in line with IEA sustainability target scenarios. Real trade-offs and consideration between economic performance and environmental footprint of the system should (and can) be taken in consideration when designing energy system. Furthermore, these trade-offs are highly scenario specific and should be assessed on a scenario-basis. Additionally, depending on the local regulatory environment, different cost & CO<sub>2</sub>eq attribution reduction can be identified. This can lead to promote adoption of such system, especially in fossil-fuel heavy (local) energy grids.

### **5.03.P-We412 Environmental impacts of photovoltaic integration in concentrated solar power plants**

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Technological advances in photovoltaic (PV) and concentrated solar power (CSP) technologies are crucial in pursuing the Green Deal's climate-neutral objective of reducing greenhouse gas emissions by at least 50% by 2030. This study introduces the developments of the CSP+ project, which designed innovative hybrid power demonstrator plants that combine PV and CSP systems. This concept innovates by integrating PV cells within the mirrors of the structure. Due to their varying solar irradiation, the pilot plants were installed in Belgium and Spain.

Using the Life Cycle Assessment methodology, the preliminary assessment of the environmental impacts of the CSP+ plants was conducted from cradle-to-grave. The functional unit is the production of 1 kWh of electricity and 1 kWh of heat. A multi-physics simulation was used to simulate the production of heat and electricity. The impact assessment used the Environmental Footprint 3.1 method, focusing on climate change and abiotic depletion categories.

The results showed that PV cell production was the greatest contributor to climate change impacts (about 51% of the total impact), mostly due to electricity consumption in their manufacturing. The second largest contributor was derived from the structure of the plant due to the steel use (47%). For abiotic depletion, the largest contributor is the plant structure due to steel consumption (60%). For the absolute impacts, the plant in Spain performed 43% better in all impact categories due to the higher energy production derived from higher solar irradiation. It was shown that the environmental impacts can be optimized according to different production levels of heat and electricity in the plant.

The CSP+ plants present an innovative technology for renewable energy generation that allows heat and electricity production in a combined plant, reducing the total impacts compared to benchmark alternatives due to the integration of PV and CSP. The results addressed the trade-offs of the system's environmental performance based on its flexibility in heat and electricity generation towards better recommendations for decision-making for renewable energy production. In addition, the innovative technologies developed in the CSP+ plants supported economies of scale through plant integration and reduced land use, which will be further investigated in a life cycle costing assessment.

### **5.03.P-We413 Feasibility of Using Alternative Fuels in Steel Industry**

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Steelmaking is a critical sector responsible for 5-7% of global CO<sub>2</sub> emissions. As a result, it has become a focus for sustainability efforts, with ongoing research aimed at reducing the industry's carbon footprint. In a blast furnace, coal in the form of coke is used as fuel and a reducing agent to reduce iron ore to iron. This study explores the feasibility of replacing traditional coal fuel in blast furnace ironmaking with alternative fuels such as subcoal, charcoal, and wood pellets. A risk assessment methodology evaluates each alternative fuel's efficiency, environmental impacts, and fuel economics to identify an efficient, sustainable, and cost-effective option.

The results of this study indicate that co-firing coal with charcoal at a composition of 20% coal with 80% charcoal is the most feasible alternative, followed by subcoal and wood pellets. While the conclusions are deemed reliable qualitatively, the methodology may require refinement to improve the quantitative results. Additionally, setbacks related to the use of charcoal, such as corrosion and alkaline ash deposition, must be addressed by employing low-ash charcoal. Higher charcoal prices can also be mitigated by implementing a higher carbon tax and stricter environmental legislation.

These findings can serve as a starting point for Tata Steel UK and other steel industries to initiate strategies towards achieving the Paris Agreement's goal of net carbon zero by 2050. Besides, this study emphasised the variability of alternative fuel options, specifically the potential use of subcoal and wood pellets after charcoal. These alternative fuels were identified as potential areas for further exploration in future research. In summary, this study provides valuable insights into the potential use of alternative fuels in the steelmaking industry. It highlights the need for further research to refine the methodology and address the setbacks associated with all alternative fuels. These findings can significantly contribute to developing sustainable and cost-effective strategies towards achieving net carbon zero in the steel industry.

For future work, this study will explore the feasibility of using hydrogen as an alternative fuel in the steel industry, conduct a life cycle assessment for all the alternative fuels considered, and refine the methodologies.

### **5.03.P-We414 ASSESSING ENVIRONMENTAL IMPACT AND MATERIAL CRITICALITY IN SCALING PEROVSKITE-SILICON TANDEM PHOTOVOLTAIC MODULES: A COMPREHENSIVE APPROACH**

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This study introduces an integrated approach that combines Life Cycle Assessment (LCA) and Critical Raw Materials (CRMs). The analysis aims to address challenges in scaling up perovskite-silicon tandem photovoltaic modules within the PEPPERONI project, funded by the Horizon Europe programme. Perovskite solar cells have attracted significant interest from the scientific community, primarily owing to their promising properties, including high power conversion efficiency (PCE). The focus of the study is on sustainable production, emphasizing environmental considerations, raw material scarcity, and the nascent nature of the technology.

The LCA conducted in this study will analyse the entire life cycle of the modules, extending from cradle-to-grave. Encompassing tasks such as resource extraction, material production, cell and module manufacturing, pilot line operation, field operation, and end-of-life management. Acknowledging the uncertainties inherent in low TRL and the novelty of this photovoltaic technology, global sensitivity analysis will be employed, aiding data visualization and facilitating the construction of probability distributions for the expected environmental impacts. This approach enhances the robustness of the results, considering the dynamic landscape of emerging technologies.

The material criticality, a central focus of this research, will be assessed also through a multifaceted approach considering supply risk and vulnerability to a supply disruption. Economic indicators, substitutability, geological, technical, and economic accessibility will be evaluated at each step of the supply chain. A multicriteria analysis will be performed, considering resource abundance, producing countries' distribution, price volatility, and other indicators. The objective is to establish a link between LCA and criticality assessment methodologies to concurrently analyse the environmental impact and metal scarcity associated with this technology.

The synergy between LCA and CRMs analysis is paramount. The combination offers a holistic perspective, enabling informed choices that balance environmental sustainability, economic feasibility, and material resource resilience. The integrated LCA and CRMs approach is poised to contribute valuable insights to the sustainable scaling of emerging photovoltaic technology.

### **5.03.P-We415 Environmental Footprint of Labgrown and Germinated Plant-based Meat: A Life Cycle Assessment Study**

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In the ever-evolving landscape of sustainable food production, this research delves into the transformative potential of lab-grown and germinated plant-based meats. Developed through advanced methodologies, these alternatives not only offer enhanced nutritional profiles but also exhibit a significant reduction in environmental impacts. This study underscores the critical role of Life Cycle Assessment (LCA) in evaluating the sustainability and nutritional implications of lab-grown and germinated plant-based meats.

Utilizing LCA, this research conducts a comprehensive analysis of the environmental footprints of lab-grown and germinated plant-based meats throughout their life cycles — from raw material sourcing and production to distribution (cradle-to-gate). Building upon existing research, our findings highlight that both innovative methods notably enhance the nutritional attributes of alternative meats, simultaneously decreasing their environmental impact compared to conventional counterparts.

To provide a holistic view, this study integrates insights from existing literature and primary data from actual producers and researchers, showcasing LCA's versatility as an invaluable research tool for assessing alternative meat sustainability. Beyond the immediate scope, we aim to expand upon prior studies by systematically comparing and evaluating various production methods. This approach aims to assist researchers, companies, and the industry in developing eco-friendly alternatives and fostering informed decision-making for sustainable food production. It is also important to note the limitations of the study, acknowledging potential constraints and avenues for improvement most especially in data collection and standardizing boundaries for the comparative analysis.

In conclusion, this study offers a nuanced exploration of the sustainability and nutritional dimensions of lab-grown and germinated plant-based meats, utilizing LCA as a key analytical tool. By building upon existing research and recognizing its limitations, our work seeks to inform future research directions and promote the development of sustainable alternatives within the food industry. Future research includes the application of social LCA and economic LCA (LCC).

### **5.03.P-We416 Consequential Life Cycle Assessment of Various Geographical Adoption of the EAT-Lancet Diet**

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The afri-food sector, while being responsible for a large part of environmental pollution and use of resources, is a key for transitioning towards a more sustainable system. Shifting to a plant-based diet is seen as one of the most efficient ways to mitigate one's environmental footprint. Yet, the previous statement often ignores the market effects of international trade, which likely offset the expected environmental benefits. A multi or global adoption of a more sustainable diet could actually have the predicted mitigation potential. Here, we evaluate the environmental impacts and land use changes of different scenarios of a diet shift in various regions following the EAT-Lancet recommendations for red meat and legume calorie intake, additionally assessing their contribution to EU and international targets to stay within 1.5°C. A consequential life cycle assessment approach is used with the agroeconomic model CAPRI, a global agricultural model focusing on Europe and including bilateral trades. The scenarios assessed are a multi-adoption of the diet by the EU-27 and China, by 40% of the population (i.e., regions eating the most red meat) and by the global population. Compared to an adoption of the EAT-Lancet diet in the EU-27 alone, preliminary results show that, for all scenarios, the gap between demand and supply is closing up and even, in the case of the global diet adoption, becoming almost equal. Furthermore, adopting the diet in both the EU-27 and China already brings much higher environmental benefits compared to the EU-27 alone, both in terms of domestic footprint and at a global level. Surprisingly, the environmental benefits from an adoption of the recommendations by 40% of the population and at a global level are quite similar, suggesting that a diet shift for regions the most far from the EAT-Lancet guidelines can already make significant improvements. As compared with business-as-usual, significant environmental benefits in all impact categories at a global and local level could be achieved by a multi and global adoption of the diet and will be compared to policy targets. These outputs contribute to a better understanding of policies needed to transition to a sustainable food system and of the role of producers and consumers in sustainable consumption.

### **5.03.P-We417 Inclusion of environmental impacts into the formulation of pig diets: Reducing the climate change impact of pig feed at minimal added cost**

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Agricultural farm-gate emissions are estimated at 13% of annual anthropogenic greenhouse gas emissions, with an additional 7% attributable to land use changes. The low profit margins of the agricultural sector suggest that careful consideration must be made when considering potential environmental mitigation options. Inclusion of environmental considerations during feed formulations may provide substantially reduced environmental impacts of pigs with relatively small changes in the costs associated to feed. This study aims to investigate the environmental effects of changes towards feed formulations through inclusion of the climate change as a minimization objective. The new feed formulations are achieved with relatively small changes in costs for the pig farmer, while keeping the feed formulations balanced from a nutritional perspective for the pig. A cradle to farm-gate attributional life cycle assessment (LCA) methodology was applied for the assessing environmental impacts of nutritionally balanced pig diets. The Danish feed unit for fattening pigs (FEsv) of 1.05 MJ/kg was defined as our functional unit. Given the strong interconnection between land use changes and climate change impacts, we applied multiple land use change methods when generating the new feed formulations with reduced climate change impacts. Feed formulations included options of multiple common feed ingredients used in Danish pig production for satisfying nutritional constrains (e.g. crude protein, fats, amino acids, micronutrients). The new feed formulations were all nutritionally balanced, although differed in contents of ingredients. Agrisoft was used for formulation of pig feed, while OpenLCA 2.0.3 combined with Agri footprint 6.3 was used for construction of a life cycle inventory. We used the Recipe 2016 (h) impact assessment method to provide a broad range of midpoint impact categories and investigate potential burden shift from climate change to other impact categories. The results are intended to provide knowledge on the potential reductions in climate change impacts given relatively small changes in the costs associated with feed formulations.

### **5.03.P-We418 Life Cycle Sustainability Assessment of nano-enabled PFAS (Polyfluoroalkyl substances)-free anti-sticking coating for bakery moulds**

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The European Green Deal policy ambitions identify the need for a Safe and Sustainable by Design (SSbD) approach to chemicals and novel materials. With the aim of making this operational, the JRC developed a framework for the identification of criteria for SSbD, the basis for a European recommendation, which specifically asks for a tiered approach depending on the data availability. This need has been addressed by the SSbD approach developed within the SUNSHINE project, which aims to develop and implement simple, robust, and cost-effective Safe and Sustainable by Design (SSbD) strategies for materials and products incorporating advanced multi-component nanomaterials. This approach aims to assess safety, sustainability, and functionality aspects at each stage of product development from a lifecycle perspective. This is achieved via a tiered approach that uses qualitative (Tier 1), and quantitative (Tier 2) assessment methods. Tier 1 consists of a questionnaire to assess safety, sustainability and functionality, and Tier 2 applies standard quantitative methods for sustainability assessment such as Life Cycle Assessment (LCA), Life Cycle Costing (LCC), and Social Life Cycle Assessment (S-LCA). LCA aims to quantify and compare the potential environmental impacts, LCC the potential economic impacts and S-LCA the potential social impacts. The present work focuses on the application of Tier 2 to one of the case studies within the SUSNHINE project: the Laurentia case study which is developing a nanocomposite coating composed of silica carbide and titanium dioxide (SiC@TiO<sub>2</sub>) to provide non-stick properties on its applications in bread baking trays. This innovative material is a substitute for Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)-based non-stick coatings, such as Teflon (Polytetrafluoroethylene or PTFE). The outcomes highlighted that under certain conditions, the innovative material outperforms the benchmark, while in other conditions, the benchmark performs better. The present work aims to highlight the relevance of applying Life Cycle Assessment, Life Cycle Costing, and Social Life Cycle Assessment to innovative products, since the design stage of development to support them in identifying hotspots, and to further improve the multi-component nanomaterial-material sector toward increasingly sustainable solutions.

### **5.03.P-We419 Environmental sustainability analysis of an industrial Italian Laundry Operations: a comprehensive Life Cycle Assessment**

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Industrial laundries, generating significant wastewater and posing toxicological concerns, threaten water bodies and contribute to environmental pollution. The lack of a detailed Life Cycle Inventory (LCI) on laundry detergent use compromises environmental assessments, relying on substitute products or proxy datasets. Laundries, known for high energy consumption, drive the need for a comprehensive LCI and Assessment (LCA). This paper aims to establish an LCI/LCA for an industrial laundry, offering an environmental profile based on an Italian case study.

Primary data for finalizing the LCI were directly obtained from an Italian industrial laundry, supplemented by literature, data from supporting databases (e.g., Ecoinvent 3.8), and information from the technical datasheets of detergents. The industrial laundry system encompasses the entire product supply chain, including the extraction and manufacturing of raw materials (including detergent), transportation and logistics, the industrial laundry process, wastewater treatment, recirculation packaging, and final disposal stages.

The calculated environmental profiles are based on the functional unit of 1 kg of linen washed through a standard washing cycle. The study's system boundaries cover the production stages, encompassing transportation for linen delivery and collection, raw material procurement, and the sanitization and washing processes. SimaPro 9.2 software and the ReCiPe 2016 H method were employed for the LCA study. A baseline scenario was compared with an alternative scenario introducing renewable energy technology, specifically solar PV panels.

The results indicate a total impact of 12.77 mPt, with the washing phase (4.62 mPt), ironing phase (4.29 mPt), and drying phase (1.56 mPt) being the most impactful activities. The washing phase's significant impact is attributed to detergent and washing product usage. Most impacts fall into categories such as 'Global Warming, Human Health,' 'Fine Particulate Formation,' 'Carcinogenic Human Toxicity,' 'Non-Carcinogenic Human Toxicity,' and 'Fossil Resource Scarcity.' The midpoint category with the highest impact is 'Fine Particulate Formation' at 5.18 mPt. The alternative scenario with renewable energy technology reduces the impact by 19.7%. Sensitivity analyses were conducted to evaluate the LCA model's uncertainty, focusing specifically on washing agents, raw material transportation, and energy consumption.

### **5.03.P-We420 Considering the evolution of the risk of natural and technological disasters while applying Life Cycle Assessment: a morphological analysis-based prospective method**

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Comprehensive planning and decision-making activities are essential to sustainably develop human societies in the face of uncertainty, especially for critical infrastructure. Results from various decision-support tools often aid such activities. Environmental impact assessment tools are a fundamental part of this, and Life Cycle Assessment (LCA) is among the most widely accepted tools. However, LCA is usually applied to model systems according to their average operation conditions, and

deviations are not considered in the inventory (LCI) phase. If a natural or technological disaster affects an industrial facility, its production will be hindered until it is fixed. From an environmental impact perspective, repair and replacement tasks may have non-negligible impacts. To verify this, such factors could be considered in LCA. One solution is to develop a dedicated risk-based prospective LCI module. This research proposes a methodological framework aiming to develop a model of potential LCI flows associated with the evolution of the risk of natural and technological disasters on a territory, using the General Morphological Analysis (GMA) approach as a basis for scenario development. Here, scenarios mean a set of circumstances that represent the situation-at-risk the studied infrastructure is under, considering a set of defined variables which are linked to each other.

The first phase consists of defining the problem space through four profiles: 1) The risks profile characterizes the properties of events that may occur within a given scenario. 2) The infrastructure profile describes the properties of the studied technological solution. 3) The territory profile describes the characteristics of the territory studied. 4) The trends profile describes the different global variables to support the scenario definition. With the four profiles defined, the second phase involves building the scenario universe by applying GMA to define the relationships between the profiles' identified variables and ranges of values. The third phase involves analyzing relevant scenarios to identify the effects of shocks between risk events and the infrastructure studied.

The above-mentioned method has been applied to a case study about the development of the photovoltaic sector in the Alpes-Maritimes, France, towards a 2050 horizon, which aids to identify challenges, opportunities, and limitations of the proposed methodological framework, also paving the way for new LCA-related research.

### **5.03.P-We421 Incorporating the Risk of Zoonotic Disease into Life Cycle Assessments of Animal Agriculture**

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Billions of animals are raised in agriculture worldwide for their meat, milk, and eggs as a source of food, and for their feathers, skin, and other byproducts for use in materials like clothing. To increase circularity across production systems, there is also ongoing research investigating how to utilize the molecular components of wasted side streams from animal agriculture as the building blocks for bio-based materials (e.g., utilizing the proteins from dairy whey to make aerogels). Life Cycle Assessments (LCAs) of animal-derived products are essential to understand their sustainability, and often consider environmental impacts like greenhouse gas emissions, eutrophication, and land use. However, such LCAs do not consider the impact animal agriculture can have on the emergence and spread of zoonotic diseases (i.e., diseases originally present in animals that mutate to infect humans). Different agricultural practices can increase or decrease the risk of new diseases emerging and spreading from farmed animals to people (and wildlife), with sometimes devastating consequences for humans (and biodiversity). For example, crowded conditions and low genetic diversity of farmed animals can enable rapid spread of viruses. Contact between multiple species in agriculture (e.g., chickens and pigs) can enable mixing and amplification of virus strains, and the possibility of contact with wildlife can be a vector for transmission either to or from non-agricultural animals. Against this backdrop, numerous diseases have emerged from animal agriculture in the past decades, with massive impacts on humans (as well as wildlife). For example, the H1N1 influenza virus emerged from North American pig farms in 2009, sparking the global "Swine Flu" pandemic. The aims of our study are to 1.) Identify and begin to quantify the risk factors associated with zoonotic disease emergence and spread due to different animal agricultural practices, and to 2.) Identify key knowledge gaps and methodological challenges that need to be overcome to develop a quantitative metric for zoonotic disease risk in Life Cycle Impact Assessment of animal products. Such a metric is needed to fully assess and compare the sustainability of different animal agricultural practices, and to support decision making towards a sustainable system of food and fiber generation.

### **5.03.P-We422 Determining the external costs of metals through risk indicators**

*Pia Heidak, University of Applied Science Pforzheim, Germany*

The operation of mines can create a great deal of value for society by providing raw materials, increasing shareholder value and developing local communities. On the other hand, mines typically generate environmental costs that are often borne by civil society - they are externalised. There have been few attempts to determine these costs for metal mining, either for commodities or for specific assets. However, the results could support the development of appropriate tools for internalisation and as a risk and sustainability indicator for customers and investors. In addition, the results could be useful for mining companies in quantifying environmental risks in monetary terms and in prioritising environmental activities.

By reviewing existing studies, we are able to show which environmental impacts have been included in existing attempts to determine the external costs of metals and which have not, although they are considered to be highly important. Furthermore, by using the economic value of metals rather than their tonnage as a reference, we were able to provide an indication of which external costs of metals might be relevant from an economic perspective.

Based on these findings, we are currently developing a methodology to calculate the external costs of mines depending on their geological, technical and environmental characteristics. As a starting point, we are analysing different targets for determining



external costs and combining these with different site-specific risk assessment methods to identify appropriate indicators and cost categories. This work is progressing and we would be happy to discuss the approach.

### **5.03.P-We423 Using GIS-methods in Life Cycle Methodologies: an application in two olive groves**

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Ecosystem services are benefits that contribute to human well-being: examples of these services are pollination, food provisioning, soil formation, and landscape aesthetics. Ecosystem services are increasingly included in policy decision-making, and in order to show the state of the ecosystem, it is necessary to apply quantification methods and models. Life Cycle Methodologies are a group of robust and proven methods that can help identify the hotspots where there is a decrease in ecosystem quality by assessing the entire life cycle of a product or a service. However, Ecosystem services are not included in this analysis. Some methods that can be included in Life Cycle Analysis are shown in combination with GIS data and results. The ESs analysed include “Fish and meat production capacity”, “Growth capacity”, “Production capacity of fruit and vegetables”, “Crop growth capacity”, which can be extrapolated from a LCA software like SimaPro or GaBi, “physicochemical filtration”, “soil erosion regulation”, which need the use of GIS software for their elaboration, and “pollination” which the method is recently developed. These methods were applied in two olive groves of the EU PRIMA Sustainolive project, whose objective was to increase the sustainability of the olive sector in the Mediterranean area. The two olive groves chosen have different agricultural practices: one uses agro-ecological principles such as the use of organic products, the integration of livestock, or the use of cover crops, and it is called Sustainable Technical Solutions (STS) while the other uses ordinary agriculture, which is called non Sustainable Technical Solutions (non-STs). The results show that, in general, more ecosystem services have less impact on STS olive grove than on non-STs olive grove: this is the case, for example, of pollinators or fruit and vegetable production capacity. However, for some categories, such as the Crop Growth capacity or the soil erosion resistance, the impacts are less for non-STs olive grove. From a methodological point of view, the results are sensitive to the input data: the physicochemical results, for example, show the same values for both olive farms because the input raster used for the soil data has a resolution of 1km x 1km (and this required a resampling of 100m x 100m) and both were included in the same Soil Map Unit with the same share of sand, clay, silt, and organic carbon.

### **5.03.P-We424 How to assess impacts on biodiversity of metal sources ? Providing indicators for decision-making**

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Metal mining and refining are known sources of impact on biodiversity, through land (and potentially sea) use change, pollution, and climate change. Impacts mostly occur at the two first steps of the value chain, namely ore extraction and smelting. Metal mining and refining happens in very diverse ecosystems and can have important effects on the local biodiversity in the three biotas: terrestrial, freshwater, and marine.

Building on existing literature, and based on a life cycle approach, we developed a semi-quantitative assessment method for metal sources, prioritizing the most important cause effect chains. As for the method, we i) first identified three metals of interest with diverse technical extraction practices ii) did a literature review, gathering information on main mining spots and practices, as well as on pressures on biodiversity caused by mining iii) identified the main drivers (land and water use, metal pollution) and built a semi-quantitative method based on cause-effect chain, with resulting rating on a scale from 1 (very bad) to 7 (very good) iv) completed our work with a quality assessment of the rating v) tested it on 15+ case studies.

Our method assessed impacts on biodiversity from water and land use, as well as from metal pollution and looks at terrestrial, freshwater, and marine biota. It combines the magnitude of each pressure with the receiving ecosystem vulnerability (using Global Extinction Probability combined with quality of governmental policies). We are currently testing it to a series of 15+ mining and refining sites, for copper, cobalt, and aluminum in Australia, Chile, China, and Congo (DRC). Those case studies present various challenges e.g., allocation between coproducts (in the case of copper and cobalt in DRC); scarce and incomplete data on metal pollution; specific vs generic data; aggregation of marine/freshwater/terrestrial impacts from heavy metals ...

Project is still ongoing, results of testing (impact on biodiversity and quality assessment) will be presented at the conference. As specific data on pollution and resulting pressures on biodiversity is largely incomplete, we will also shed light on quality assessment and discuss how to combine it with results to provide actionable yet robust indicators for business decision-making.

### **5.03.P-We425 Measuring biodiversity impacts of salmon production: A case study for Norway**

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Aquaculture is an alternative protein source to land based farming for protein production and Norway is one of the leading countries that produce and export farmed salmon across the world. with a share higher than 50% in 2020. The production is expected to increase due to climatic suitability and economic development. While it creates value for Norway, the environmental impacts of the production need to be evaluated to mitigate the development of future strategies and to facilitate

decision making. There are many studies investigating the different aspects of aquaculture industry to improve the current practice. In terms of environmental impact assessment, currently available research for Norway mainly focuses on the evaluation of lice treatment options and climate change impacts. This study aims to present the impacts of aquaculture on biodiversity, by focusing on two production regions (North and South Trøndelag) to evaluate the potential differences at fine scale.

In this study, ex-post environmental impact assessment was conducted for the salmon production in Trøndelag, Norway for years between 2018-2021. The examined production processes include growth stages from hatchery to full grown salmon (grow-out) to be transported to slaughterhouse. In order to quantify the impact, functional unit was determined based on the weight of final stage of the system boundary, considering the unit grow-out mass (one kilogram) and impacts are quantified accordingly. The inventory data for the assessment was obtained in collaboration with the county municipality.

Environmental impacts were quantified by utilizing LC-Impact methodology for relevant impact categories: climate change, land use, water use, ecotoxicity, eutrophication/acidification. The impact values were calculated for each year separately to evaluate the temporal changes. Also, as lice is the main threat for the mass produced, different treatment options were evaluated to measure the environmental performance of each treatment option. The results of the study were utilized to investigate the synergies and trade-offs between treatment options and biodiversity conservation.

### **5.03.P-We426 Moving forward with the definition and assessment of positive biodiversity impact from financial investments using an LCA-based methodology**

*Ana Judith Guerrero Esquivel, PRe Sustainability, Netherlands*

Having an extensive biological diversity on earth is essential for the life of the planet. However, due to extensive deforestation (among others), more than one million species are threatened with extinction [1]. Under this scenario, it is necessary to limit activities that represent a threat to different ecosystems and promote those who can have a positive effect. The financial sector plays a crucial role on deciding which of these type of activities is executed, as this sector decides where money is invested or not. Thus, it is essential to bring biodiversity into the financial equation. The increasing regulatory pressure of the European Green Deal [2] and more, makes the protection and restoration of biodiversity a cornerstone of green finance.

The Biodiversity Footprint for Financial Institutions (BFFI) methodology, a method grounded on LCA, allows to quantify the biodiversity impacts of diverse economic activities. This year, for the first time, the BFFI methodology was applied to the ASN Biodiversity Fund which aims to create a positive impact by investing in activities aiming at restoring and protecting biodiversity [3] such as nature conservation areas [4] or agricultural projects with sustainable farming practices [5].

Applying the BFFI methodology in this context required defining a clear framework for what is positive and avoided biodiversity impact. Additionally, it required developing new approaches to include activities not assessed before with the BFFI methodology. As a result, a proposal was done on how to model investments in projects focused on: (1) Agroforestry & sustainable agriculture; (2) Land conservation; (3) Reforestation; and (4) Aquaculture. Additionally, a first assessment on these type of investments was performed.

These developments within the BFFI methodology and the subsequent first assessment of the ASN Biodiversity Fund contribute to understanding how investments in restorative projects could have a 'positive' impact on nature.

**References** [1] <https://www.un.org/en/climatechange/science/climate-issues/biodiversity>

[2] [https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal\\_en](https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal_en)

[3] <https://beleggingsfondsen.asnbank.nl/fondsen/asn-biodiversiteitsfonds.html>

[4] <https://www.worldbank.org/en/news/press-release/2022/03/23/wildlife-conservation-bond-boosts-south-africa-s-efforts-to-protect-black-rhinos-and-support-local-communities>

[5] <https://www.slmpartners.com/europe>

### **5.03.P-We427 Integration of environmental sustainability for decision support applying the SSbD strategy at the design stage of nanomaterials production**

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Sustainability is one of the main cutting-edge topics of the 21<sup>st</sup> century, which the United Nations has defined as “meeting the needs of the present without compromising the ability of future generations to meet their own needs”[1] and to put it into practice, 17 United Nations Sustainability Development Goals (UN SDGs) were presented in 2015 as part of the 2030 Agenda for Sustainable Development. Life Cycle Assessment (LCA) is one of the most powerful tools for measuring the environmental impact of products (NMs and nanoproducts) over their entire life cycle, from the extraction of the raw materials, manufacture, distribution, use and waste disposal, and underpins progress towards the UN SDGs and supports the European Green Deal.

Over the past decade, the increasing development and production of nanomaterials (NMs) and nanoproducts and their widespread usage in a variety of industries including food, cosmetics, medicine, pharmaceuticals, etc. have led to associated negative effects on the environment. As an area of high innovation, NMs and nanoproducts pioneered anticipatory assessment of safety at the early stage of design (so-called Safe(r) by Design), and thus offer an excellent case study for integration of sustainability also at the design stage, to achieve the goals of Safe and Sustainable by Design (SSbD).

To further embed sustainability assessment into the production and use of NMs and their associated products, identification of the hotspots of environmental impact during the NM and nanoproduct design phases, application of the Safe and Sustainable by design (SSbD) approach, in which LCA is embedded, will offer new insights. Coupling these with the application of the principles of green chemistry and green engineering should dramatically lower the environmental footprint of NMs and nanoproducts, whilst maintaining the desired functionality and safety profile. This poster demonstrates the application of this integrated approach to existing NMs synthesis routes, using the example of silver NMs applied in personal care and medical products. The output is a decision tree, implemented as a web application, to support SSbD of NMs and nanoproducts through optimisation of synthesis reactions and conditions via green chemistry and engineering principles.

[1] <https://www.un.org/en/academic-impact/sustainability>

### **5.03.P-We428 Integration of Robust Chemical Safety and Sustainability Assessment with Agent-Based Models Applied to the Energy Transition**

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The energy transition is a complex and dynamic process that has the potential to bring about significant environmental and social changes. The shift towards renewable energy sources may lead to a reduction in greenhouse gas emissions and air pollution, but it may also have unintended consequences such as land-use conflicts, water scarcity, and negative impacts on biodiversity. Life Cycle Assessment and Ecological Risk Assessment are powerful tools to identify possible unintended consequences. However, these assessments are inherently uncertain and do not take into account the complex network of actors involved in the energy transition, such as the government, companies, and citizens. Therefore, it is challenging to extract robust policy advice from these assessments. The objective of this research is to integrate existing knowledge on assessing chemical safety, human health, and sustainability impacts with techno-economic and behavioral modeling to offer a broader understanding in steering towards a more resilient energy transition. This integrated modelling approach has the potential to better explore the different dimensions of uncertainty linked with the complexity of the system, expose the emergence of unexpected consequences, and inform decision-making processes that account for the diversity of perspectives, values, and preferences in the transition towards a sustainable energy system.

To demonstrate the potential of an integrated modeling approach we examine a fundamental aspect of the energy transition: residential thermal comfort. We propose practical computational strategies to integrate Life Cycle Assessment and Ecological and Human Health Risk Assessment with Agent-Based Models applied to residential thermal comfort in the energy transition. Our approach incorporates uncertainty, social behavior, environmental and human health impacts in a probabilistic integrated model in an effort to embrace the complexity of energy transition-related challenges and gain insights from the integration of different perspectives and methods. At the same time the integration of such models enables us to leverage state-of-the-art methods in e.g. Global Sensitivity Analysis to largely reduce the complexity, streamline analysis and interpretation, and thus to apply such complex modelling for informing the decision-making process of policy makers.

### **5.03.P-We429 Integrating LCA and MFA with Linear Programming: Assessing Pathways of Energy Storage within the European Union**

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To fulfil its net zero promise by 2050, Europe shall replace most of its passenger car fleet with only Zero emission vehicles (ZEV). Replacing the 270 million passenger cars with ZEVs, currently, battery electric vehicles (BEVs) are the most promising technology. The embedded energy storage system (ESS), will drive raw material (RM) demands and the RMs used currently (e.g. lithium, cobalt, nickel etc.) are known but in the future new chemistries involving new RM are to be expected.

How much cobalt will be needed in the EU in 2035 if cobalt chemistries are losing their domination? Could we have, under given technological evolution, an environmental advantage of recycling batteries earlier than their end of life?

In this work, the Python framework combines environmental life cycle assessment (E-LCA), material flow analysis (MFA), and linear programming (LP). This model traces the material flows, primary and secondary, and quantifies and locates their impacts within the value chain. The integration will be operationalized using Python packages Brightway, Pyomo, lca\_algebraic and HIGHS.

More precisely, bills of materials and LCA impacts of modelled processes will be accounted for in the optimisation model and used as the objective to minimize environmental impacts (EI) or RM consumption and within the set of constraints to enforce environmental targets or maximum capacity (e.g. Gigafactories built in 2030). We represent each process (i.e. in our case, extraction, refining, active material, cell manufacturing, battery manufacturing, use phase and recycling) as one decision variable (DV) per year, i.e. the production output (e.g. production per year). Each DV is linearly associated with their respective EIs. Stocks are also modelled (e.g. Use phase, dissipative losses). The processes are impacted over the years by the power supply mix and, hypotheses on battery technology or ore grade.

The results of this model give us the primary and secondary RM needed per year, the production volume and EIs per process.

These results will allow a future assessment of the security of supply, the benefits of circular economy strategies, and the impacts of key parameters on circularity or EIs. Eventually, this model should be able to support decisions considering various key performances of the overall systems.

Disclaimer:

Views expressed are those of the authors and do not reflect an official position of the European Commission.

### **5.03.P-We430 Development of a Novel ‘Hub & Spoke’ Framework for the Holistic Sustainability Assessment of Chemical Value Chains**

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Sustainability assessment is a multifaceted research field, encompassing not just environmental considerations but also the interplay between economic and social dimensions. Current literature highlights the pressing need for a comprehensive approach that integrates these three ‘strands’ seamlessly. To date, research has focussed predominantly on isolated assessments—environmental, economic, and social—failing to capture both their interconnections and burden shifting. This fragmented approach to assessments leads to an incomplete understanding of problem spaces, potentially catalysing erroneous investment and R&D efforts.

The primary challenge lies in rectifying the remaining misalignments between environmental, economic, and social assessments. Social impact characterisation models, analogous to those seen in environmental studies, have consistently eluded practitioners, a consequence of the complexity and cultural specificity of impact pathways. To tackle this issue and deliver quantified and repeatable assessment results, a set of seven risk-based characterisation models has been developed as part of a proof-of-concept exercise; at completion, results are fully defined for 129 countries. In this, the social risk is quantified through the consideration of each impact indicator’s stimulating and de-stimulating factors.

Integration of these social characterisation models within holistic assessments has also been demonstrated through a case study examining soda ash value chains in Asia. All three strands are shown to be aligned with the ISO 14040 standards, delivering the first fully quantified and integrated holistic sustainability assessment. Multi-criteria decision-making (MCDM) techniques are also employed in the form of hybrid AHP-TOPSIS, systematically aggregating impact indicator scores in parallel to the traditional objective assessment, delivering a bespoke ranking of the available value chain permutations.

The integration of these three strands holds significant promise, not only providing a more nuanced understanding of value-chain sustainability but also setting the stage for robustly informed industrial decision-making. By harmonising these assessments, organisations and institutions can realise a more strategic approach to sustainable development, quantifiably accounting for the interrelations between environmental preservation, economic viability, and social justice.

### **5.03.P-We431 Circular economy metric for the energy transition: a life cycle thinking approach**

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The circular economy will play a key role in the energy transition pathway undertaken by the European Union (EU) in particular in decoupling the decarbonization of electricity generation from the demand for primary raw materials essential for renewable technologies. Planning effective strategies requires measurement systems to establish the position of the current system and monitor the progress towards quantifiable circularity objectives. Within such a framework, in the present work, the authors present a metric to measure the circularity of the electric generation system at the national level (macro-level analysis). The metric was developed based on a circular economy definition and principles customised for the electricity generation system, and it includes indicators related to policies supporting circularity, materials, energy and waste flows and specific indicators related to environmental sustainability, estimated applying the Life Cycle Assessment (LCA) methodology.

The identified indicators are clustered under four areas of analysis, “enabling factors”, “configuration of the electricity power plants and operational phase”, “end of life management” and “improvement of the environmental life cycle impacts”.

The metric provides a synthetic circularity index through the aggregation of the scores assigned at each indicator based on a system of weights obtained according to the analytic hierarchy process. The circularity index can range from 0 (linear) to 1

(circular) and classifies the electricity generation system into four levels of circularity: “beginner”, “started”, “concerned” and “circular”.

The framework of indicators will be applied to the Italian electricity generation system in 2020 providing a baseline for tracking the progress towards a more circular energy system. In addition, from a monitoring perspective, a first application referred to 2021 and 2022 will be proposed. According to the Bellagio Declaration the EU and National official statistics, policy information, but also other information sources beyond official statistics will be used for indicators assessment.

The proposed framework is suitable for application to other Countries and can support the decision makers in planning strategies aimed at increasing the circularity of the electricity generation system at the macro scale identifying potential areas of improvement and providing reliable information for the allocation of the available financial resources.

### **5.03.P-We432 An Integrated Framework for Combining Environment, Health, and Sustainability Metrics: Zinc Case Study**

*Eric Van Genderen, International Zinc Association, International Zinc Association*

Increasing requests from stakeholders across mineral value chains demand information on various aspects of material composition, risk to health and environment, and emissions footprint. As a result, industry strategies to support measurable progress towards zero pollution and decarbonization are becoming increasingly sophisticated. While individual companies take specific measures to address customer and jurisdictional requirements, industry sectors are integrating decades of research and development to identify metrics and pathways towards achieving decarbonization and reducing environmental emissions. The development of credible systems for assessing life cycle impacts (ISO guidance, GHG Protocol) or chemical management (GHS CLP, risk assessment) considerations for products have been individually challenging. However, consumers now demand a full spectrum of Environment, Social, and Governance assurance from producers. Using decades of research from the zinc industry on material flow analysis and life cycle assessment (LCA), decarbonization scenarios were developed. In an additional step, environmental emission scenarios from zinc production and use (e.g., EU REACH dossier) were used to link source apportionment with risk assessment and abatement scenarios. A synergistic result of the analysis illuminated zinc’s “value in use” that can equate to massive intractable carbon emission reductions from steel and concrete sectors. Results demonstrated that an integrated framework for combining LCA and risk assessment enables innovation. This presentation will offer a framework assessing the spectrum of benefits and impacts for materials along a value chain.

### **5.03.P-We433 Sustainable future of peatlands: evaluation of peat extraction site restoration strategies from a life cycle thinking perspective**

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Peatlands are a vital part of the ecosystems that have a critical role in carbon storage, preservation of biodiversity, and flood risk reduction. The environmental challenges posed by the industrial extraction of peat include an increased risk of a large amount entering the atmosphere with GHG emissions, mainly CO<sub>2</sub>, N<sub>2</sub>O, and CH<sub>4</sub>, over a long period of time. Latvia is one of the European states with the highest percentage of relative area cover of peat and peat-topped soils accounting for 1.2 million tons, representing 31% of horticultural peat used in the EU. The financial benefit of peat exports from Latvia has increased from 104 million euros in 2012 to 293 million euros in 2022. Several international policy planning documents have been issued requiring Member States to implement restoration measures for the peat extraction sites. However, it is not clarified how exactly to find and implement the restoration measures without having an adverse economic from closing peat extraction industry and social effect on communities involved. Therefore, this study aims to develop a novel evaluation framework for evaluating possible peatland restoration strategies in peat extraction sites within the three pillars of sustainability, environmental, economic and social. The framework is implemented in a quantitative assessment tool, which addresses the sustainability evaluation of restoration strategies for peat extraction sites in the form of a multi-criteria analysis within a life cycle thinking perspective. The selected indicators for life cycle sustainability assessment include environmental impacts, life cycle costing, social impacts of restoration strategies. The tool is tested and validated within local case study on existing peat extraction site. The results of the study show a valuable insight into the most sustainable peat restoration strategies for the extraction site managers and also, contributing to advising the policy planning for the implementation of the peat extraction site restoration measures based on scientifically sound methodology.

### **5.03.P-We434 Portfolio Sustainability Assessment incorporating ProScale for a holistic view on human toxicity potential**

*Peter Saling<sup>1</sup>, Peter Kölsch<sup>2</sup> and Wibke Lölsberg<sup>2</sup>, (1)CDS/S, BASF SE, Germany, (2)BASF SE, Germany*

The Portfolio Sustainability Assessment, TripleS, was introduced at BASF with the aim of increasing our portfolio of innovative and sustainable solutions and the sustainability performance of the value chains we serve. By assessing key drivers and issues in our customers’ industries, we strive to identify the sustainability contribution of each of our products in its specific application. To do so, solutions in their respective application and region are assessed in terms of defined sustainability criteria.

The results derived from the evaluation of the value chain support business units by given them a clear picture of the sustainability drivers and concerns in their current and future portfolio. In this respect, the performance assessment serves as an early warning system. We aim to identify at an early stage those solutions that are likely to be affected by regulations and/or a negative market perception. Where deemed necessary, mitigation options for solving the sustainability issue(s) are developed. In addition, the TripleS tool serves as a steering instrument to trigger innovations that ensure differentiation in the markets through their contribution to sustainability and improved quality of life. Consequently, BASF has derived measures to use TripleS as a strategic steering tool for its portfolio development. The two segments of most sustainable products, Contributor and Pioneer, were defined as the basis. Through this KPI, the qualifiers for such most sustainable products are an integral guidance for our business development with the goal to grow this share of the business over proportionally.

To further enhance the assessment ProScale can be used to evaluate the human toxicity potential of substances in their application across the full lifecycle. The methodology allows to assess application specific exposure potential arising from direct exposure of hazardous substances in a product along its entire life cycle. Proscale scores can be calculated at different levels of aggregation, e.g., for an entire life cycle, a part of a life cycle or a specific unit process. A practical example will be presented.

### **5.03.P-We435 Communicating life cycle global health impacts of addressing the plastic pollution crisis**

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Human health risks associated with emissions across the plastic value chain are a growing concern but limited quantitative evidence has been available to inform international decision-making. We combined Life Cycle Assessment with Material Flow Analysis, using the Plastics-to-Ocean model, to estimate the human health impacts associated with scenarios of global plastic management in 2040. Scenarios included: (1) *Business-as-Usual* (BAU) projections for plastic production, management, and waste, and (2) *System Change* (SCS) comprising reduced global plastic production, increased waste collection and disposal, recycling, reuse, and material substitutions. We applied Life Cycle Impact Assessment (LCIA) methods with characterisation factors for estimating Disability-Adjusted Life Years (DALYs), including 15 combinations of modelling assumptions, perspectives, and time-horizons from ReCiPe 2016, Impact World+, LC-Impact and USEtox. We compared estimates between methods to evaluate implications for interpretation, communication and decision-making in the context of plastic waste reduction and global health. In 2040, the BAU global plastic system was associated with between 58,000 and 120 million years of healthy human life lost worldwide, the burden of which was reduced by 2% – 83% in SCS, depending on the LCIA method. Climate change was an important driver of health impacts in both system scenarios (BAU: 6.80E+05 - 2.23E+06 DALYs, SCS: 3.78E+05 - 1.24E+06 DALYs, 100-year time-horizon). Climate-related DALYs were influenced by whether models included heat stress-induced cardiovascular disease and rising malnutrition alongside the health burdens of diarrhoea, malaria, and flooding risk. Though all LCIA methods showed virgin plastic production as the leading source of health impacts in 2040, we found significant differences in terms of the relative importance of climate-related DALYs versus reduced water availability leading to malnutrition. Different time-horizons, perspectives and inclusion of environmental stressors were associated with the scale and severity of health impacts associated with global plastic scenarios. Careful interpretation and communication of human health impacts is critical to informing decision-makers, ensuring health concerns are considered in policy and monitoring, and that measures are used appropriately and transparently in terms of their validity, precision and remaining ‘known unknown’ and ‘unknown unknown’ risks for global health.

### **5.03.P-We436 On the Readiness of Social Life Cycle Assessment for Integration in Life Cycle Sustainability Assessment and Safe and Sustainable by Design Frameworks**

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Social Life Cycle Assessment (S-LCA) faces fundamental methodological barriers. Its increasing applicability, e.g., in frameworks as Life Cycle Sustainability Assessment (LCSA) or Safe and Sustainable by Design (SSbD), calls for a critical evaluation of S-LCA and its feasibility to adequately assess the social pillar within these product-centric frameworks. We explore key differences between general S-LCA impact assessment methods and evaluate the value-added of S-LCA in its current form, compared to more qualitative and organization-oriented approaches to assess social impacts over a product's life cycle.

Using the 2020 UNEP Social LCA Guidelines as a starting point, with a particular emphasis on Reference Scale approaches, we compare five methods for evaluating social impacts of a textile garment. Two methods are based on the PSILCA social database (through datasets with and without an activity variable as scaling factor). Third, the Subcategory Assessment Method is evaluated as a more organization-centric and semi-quantitative approach to S-LCA. As fourth and fifth, two more qualitative social due diligence approaches, based on the OECD Due Diligence and UNGP Principles for Business and Human Rights and IFC Performance Standards, are evaluated

The absence of a standardized S-LCA approach fosters a problematic variety in methods being developed. Evaluating five methods highlights how each handles very different modeling structures, whereby results differ considerably in nature, length, and social topics covered. Product-oriented S-LCA, which can be performed using social databases, faces important limitations in being restricted to assess quantifiable aspects only. More qualitative and organization-centric approaches assess the social performance of entities within a product's value chain rather than establishing cause-effect relationships between processes comprising the product system and social indicators. Such organizational approaches can rather be used to conduct social due diligence of suppliers in the target organization's value chain.

We conclude that a product-focused approach for S-LCA using a functional unit and limited to including quantitative indicators only is best for integration into LCSA and SSbD. These assessments can be complemented by more qualitative approaches assessing value chain actors. We call attention to the equal importance—but fundamental difference in approach—between product-based S-LCA and organization-level assessment.

### **5.03.P-We437 Assessing Social Life Cycle Impacts of Power Technologies in the Spanish Electricity Mix**

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Power generation systems are at the forefront of national energy transitions in many countries, such as Spain, which stands as a notable example. However, this profound transformation could have multifaceted implications, leading to unintended consequences on society. Under these circumstances, the present work aims to shed light, for the first time, on the social impacts of the Spanish power sector and their supply chains by using the social life cycle assessment methodology. The functional unit is defined as 1 kWh of electricity produced in Spain in 2022, distributed across the following technologies: 23% natural gas combined cycle (NGCC), 23% onshore wind, 21% nuclear, 10% solar photovoltaics (PV), 7% cogeneration, 7% hydro dam, 3% coal, 2% concentrated solar, 2% biomass, 1% hydro run-of-river, and 1% incineration. A cradle-to-gate approach is taken encompassing the production of operational inputs (e.g., fuel or energy), operation and construction of the power plants, manufacturing of capital goods as well as extraction and refining of raw materials. To compile the social life cycle inventory, data collection encompassed: i) the identification of national suppliers along the value chains of power technologies, and ii) the quantification of working hours linked to each process within the predefined system boundaries. All of this information was integrated into the PSILCA database to ultimately derive the social profile of the Spanish electricity mix. Although the results in terms of four selected indicators (child labour, contribution of the sector to the economic development, frequency of forced labour and women in the sectoral labour force) reveals significant differences, three main social hotspots emerge from the analysis: i) the production of solar PV panels in East and Southeast Asia (solar PV plants), ii) the extraction and refining of natural gas in North Africa (NGCC and cogeneration plants), and iii) the construction and operation of hydro and nuclear plants in Spain. In the first two instances, impacts directly relate to the risk levels associated with the involved processes, while in the latter case, a relatively high number of working hours lead to the significant contribution of these technologies. Therefore, high renewable production in national electricity mixes doesn't guarantee a positive social performance, revealing the need of balanced environmental and social considerations in energy policy making processes.

### **5.03.P-We438 Proposal of a scalable product biodiversity footprint framework based on state-of-the-art methodologies for eco-design of cosmetic products**

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Today, within the sustainability program "L'Oréal For The Future", ambitious commitments have been set by the company to continue tackling environmental issues on climate change, water, resources as well as biodiversity. The tool to support product developers in contributing to these commitments is SPOT (Sustainable Product Optimization Tool), an eco-design tool for cosmetic products based on life cycle assessment. As the science around biodiversity footprinting continues to progress, it is essential for L'Oréal to develop an understanding of the biodiversity footprint methodologies at product level to support eco-design with aim of the preservation or even the promotion of biodiversity as well as to explore how it connects to commitments at corporate level.

In order to leverage the already, acquired knowledge and available life cycle inventories and methodology, the LCA-based approaches are prioritized when building a relevant product biodiversity footprint framework. A panel of methods have been reviewed and selected by L'Oréal based on existing literature. A product biodiversity footprint framework built on the state-of-the-art methodologies has been then proposed. It suggests i) the use of the current SPOT method for a first screening level of insights and ii) use of additional known LCIA methods in biodiversity footprint studies to provide more relevant information and results when needed. This framework is currently being tested and assessed.

This work will enable L'Oréal to formulate recommendations for strategic deployment and implementation of product biodiversity footprint assessment for different use cases :

- At-scale and streamlined biodiversity footprint screening studies, with key data and hypotheses, which could be applied to the entirety of L'Oréal product portfolio as well as innovation projects at different life cycle stages (sourcing, ingredient production, processes, end of life, etc.).

- Specific biodiversity footprint studies, with more refined and accurate data, applied to some products or relevant projects.
- Complete and robust studies, with data and hypotheses of high quality as well as peer-reviewed steps for a potential biodiversity claim at the product level.

A brief discussion on the articulation with the corporate biodiversity footprint metrics will also be presented to highlight the relevance of the proposed approach.

### **5.03.PC LCA and Beyond – Integrating Sustainability and/or Other Dimensions for a More Informed Decision-Making**

#### **5.04.A LCA in Policy, Decision-Making and Communication to Support the Transition Towards Sustainable Consumption**

##### **5.04.A.T-01 Environmental Footprint for product policy support: current status and challenges**

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The use of Life Cycle Assessment (LCA) in EU policy has increased over the last decades with life cycle thinking becoming central for the European Green Deal ambitions. In particular, EU product policy development has benefited from the systemic approach of LCA in order to enhance the environmental performance of products while preventing trade-offs among environmental issues or life cycle stages. In this context, the European Commission recommends the Environmental Footprint (EF) to ensure the robustness and comparability of environmental information provided in the EU market.

This work aims to review the current use of EF methods in EU product policy, while discussing advantages and challenges of the LCA implementation. In addition, further developments for the EF are explored. The analysis focuses on the following policy examples: EU Green Public Procurement (GPP) and Product labelling for environmental information. Other examples may include Ecodesign for Sustainable Products Regulation (ESPR) proposal or the Batteries regulation.

The EF method can support policy-making in different steps of the policy cycle. Examples during policy design includes the use of EF data for defining criteria or requirements. In the implementation of policies, the EF can support the development of methodologies to provide a robust and comparable framework, e.g., to inform consumers about the environmental impact of products. For policy monitoring, EF-based indicators or frameworks can be developed to support the quantification of the effects of product policy. While several advantages of the use of EF in policy support are identified, the EF faces some challenges associated to the current modelling requirements (inventory) and impact assessment methods. Most of these challenges are common to LCA and aligned with current discussions and advancements in the LCA community.

##### **5.04.A.T-02 The Green Deal's New Call for Greenhouse Gas Data: A Daunting Task or Déjà Vu?**

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To achieve the European Union (EU) overall net-zero emission target until 2050, the European Green Deal announces a variety of new reporting regulations pressuring companies to report greenhouse gas (GHG) emissions of their activities, assets and their organization as a whole. Therefore, companies do not only struggle to keep up with the latest reporting requirements but also experience increasing difficulties to manage through the jungle of accounting methodologies and data sources. This leads to an increased accounting workload as well as to methodological and practical accounting errors negatively affecting the reporting quality [1].

To this end, our study aims to identify data which is required within the scope of two or more reporting schemes. We hypothesize that the in-depth theoretical knowledge of these data synergies could significantly reduce the associated accounting workload and simultaneously increase the consistency of reported results. As a practical outcome our analysis shall serve as the basis of future GHG accounting and data management concepts in companies.

To validate our hypothesis, we perform a three step analysis: first, we perform a systematic review of current GHG disclosure obligations within public regulations, introduced in the past and within the European Green Deal. Second, we review and cluster existing GHG accounting methodologies and identify which methodology is used within a certain reporting regulation. And third, we characterize data sources needed to be consulted within the respective accounting methodology. Synergies are identified and discussed regarding their methodological relevance and their practical potential to reduce accounting workload.

Our results show that similar data is used across system boundaries for varying reporting obligations. Being aware of these data synergies indicates that new reporting obligations might not necessarily come with new accounting challenges. Instead, reporting companies should make use of these synergies to recognize common data sources, thus avoiding multiple



consultation of the same data. This way, corporate GHG accounting could substantially be improved, while reducing the accounting workload.

[1] Klaaßen L, Stoll C. 2021. Harmonizing corporate carbon footprints. *Nat Commun* 12, 6149 <https://doi.org/10.1038/s41467-021-26349-x>

#### **5.04.A.T-03 Eco-labelling in France, time has come for implementation and scale up !**

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France is on the verge of finalizing an extensive ecolabeling initiative, beginning with food and textiles, initiated in 2009. This effort aligns with the "French Climate and Resilience law," responding to citizens' calls for improved environmental information. Amidst the emergence of many private initiatives, France positions itself as a "pilot country" in the EU. ADEME and the French Environment Ministry propose a voluntary ecolabeling scheme in 2024, set to become mandatory in 2025.

The ecolabeling methodology centers on Life Cycle Assessment (LCA), presenting a PEF-wise approach. While rooted in the PEF methodology, adjustments address critical limitations. Modifications include modification of toxicity indicators and weighting. An "ecosystem services module" is introduced, addressing some biodiversity aspects lacking in PEF. The textile sector integrates a microfiber indicator.

Operationally, a tiered approach allows flexibility in data collection, accommodating stakeholders' capacities. Background data relies on established databases for food (Agribalyse) and textiles (Base Empreinte). An open-source Ecobalyse tool supports method development, implementation, and ensures transparency in stakeholder discussions.

The success of the French ecolabeling scheme hinges on achieving acceptability across multiple dimensions: methodological robustness, operational feasibility, and stakeholder support. The proposal introduces a "cost for the planet" concept, utilizing a dedicated unit for environmental impact (mPt). In contrast to the A-B-C-D-E scale used by most private schemes, this approach emphasizes that every product has an environmental impact, addressing ecodesign, but also consumption levels and sobriety dimensions. It avoids complex categorization debates, threshold effects, and aligns with consumers' familiarity with comparing products based on financial costs.

The French ecolabel format, currently under development, will be presented at the SETAC conference. The scheme aims to provide clearer and more comprehensive environmental information to a broad market, bringing LCA knowledge into the households of millions of French citizens. It acknowledges LCA's limitations and proposes short-term fixes until academic consensus is reached, and LCA databases are more comprehensive. The communication and pedagogical aspects are deemed crucial, given the lack of shared understanding on environmental metrics and diverging interests among stakeholders.

#### **5.04.A.T-04 Harmonization of LCA methods, rules and guides across sector boundaries: A shared responsibility of science and industry.**

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Several scientific-industrial initiatives are working on guides or rules to sharpen requirements of Product Category Rules (PCR), LCA sector-guides and method specifications. All initiatives aim for collaboration among the entire value chain. Environmental Product Declarations Systems (EPD), Together-for-Sustainability (TfS), the Automotive Network Catena-X, Clean Vehicles Research (CLEVER), Sustainable Apparel Coalition (SAC) are examples. Whereas requirements are based on ISO, the initiatives aim sector-specific rules, beyond ISO. Goal is to define sector consistent rules and specifications enabling result reliability based on technological or supply chain differences, free of (hidden) methodological differences. Everybody in the supply chain (resource extractor, supplier, manufacturer, B2B customer, final user, recycler as well as policy maker) is expecting reliable results based on (real or natural) technological and supply chain differences, rather than (virtual or rule made) methodology. The aim sounds too well to be true. The sector initiatives are appreciated and supported. However, there is an issue, which is widely disregarded (or at best acknowledged and postponed), which can only be solved via constructive and responsible cooperation of involved industry, science and policy: Neither LCA nor the supply chain are sector-specific. The presentation will demonstrate concrete (and wherever possible quantified) practice examples where "sector specific rules" need to be harmonized into "intersectoral rules". Method requirements (e.g. by-product treatment, end-of-life assignment, data quality indication, life-cycle system boundary allotment, energy system choice, regionalization and technology specifications, data gaps handling) to improve results and consistency, need to be accepted by "providing or receiving neighbor sectors". We present quantitative figures concerning the (real) impact of certain differences in key method choices; based on overall LCA results of key products. It is aimed to provide more clarity on where existing general claims "like allocation is very important" should be specified into "allocation is important in these specific circumstances". The presentation provides information and background for future constructive and responsible cooperation of all involved stakeholders of industry, science and policy towards harmonized LCA application, rules and guides across sector boundaries, based on physical and practical evidence.

#### **5.04.A.T-05 Combined Assessment of Absolute Sustainability performance and Life Cycle Damage of global consumption**

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LCA has been developed for determining impacts of products and services over their entire life cycle, assessing damages on human health (HH), ecosystem quality (EQ) and natural resources, with a focus on relative improvements of products. In parallel, Absolute Environmental Sustainability Assessments (AESAs) assess the absolute sustainability performances of products and compare their impacts with limits fixed by planetary boundaries. Both frameworks continue evolving separately and there is a need for combining these approaches for sound decision making.

This study presents a framework to consistently perform a combined assessment of absolute sustainability and damages, to be simultaneously considered by decision-makers, and apply it to evaluate the impacts of world overall consumption. Using an Environmentally Extended Multi-Regional Input-Output Model to evaluate elementary flows associated with global consumption, Impact World+ to assess the corresponding Life Cycle Damages, and sustainable boundaries aligned with the LCIA metrics, we determine damage and absolute sustainability status for 18 specific impact categories affecting HH, and EQ areas of protection (AoP). An area-based graphic, enables us to visualize for each AoP both the exceedance fold of each impact category specific planetary boundary (Y-axis), and the corresponding damages (X-axis and total area).

Damages associated with global consumption are exceeding global sustainable levels for 50% and 62,5% of the HH and EQ impact categories. The exceedance does not directly relates to the damage *per se*, and are complementary representations. For example, the global consumption exceeds climate change planetary boundary by a factor 30, with a damage of 9.2E+7 DALY/y, whereas it exceeds the fine particulate boundary by a lower factor of 10, but with a higher impact of 1.3E+8 DALY/y. Similar contrasting tendencies are observed for ecosystem quality impacts contrasting high impacts and high exceedance factors associated with land use (2.6E+13 PDF-m<sup>2</sup>-y/y; exceedance fold of 39) and climate change (2.0E+13 PDF-m<sup>2</sup>-y/y; exceedance fold of 30). In contrast terrestrial acidification is below planetary boundaries (exceedance factor of 0.7) but still has a substantial impact (4.8E+12, PDF-m<sup>2</sup>-y/y). In conclusion, it is crucial to consider both planetary boundary and level of damages, damages being able to provide science-based weighting to the respective planetary boundaries in different impact categories.

#### **5.04.B LCA in Policy, Decision-Making and Communication to Support the Transition Towards Sustainable Consumption**

##### **5.04.B.T-01 Assessing Environmental Impact Reduction Potential for Blue Ammonia: Navigating Complexities in Carbon Capture and Utilization Modelling**

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Ammonia, an indispensable cornerstone in global chemical production, accounts today for approximately 2% of the total energy demand globally, with an overwhelming share of the energy stemming from fossil-based sources like natural gas and coal. This positions Ammonia as one of the most emission-intensive commodities produced by heavy industry and prompts the need for a more environmentally friendly alternative. Emerging short-term alternatives like Blue Ammonia use steam methane reforming with natural gas as a hydrogen source while simultaneously capturing and either storing or utilizing carbon dioxide (CO<sub>2</sub>) emissions, presenting itself as a climate-friendly option. With more Blue Ammonia suppliers opting for CO<sub>2</sub> capture and utilization (CCU), the key question arises: which standards and guidelines must be followed to ensure a transparent representation of environmental impact reduction? This research focuses on assessing and discussing the most up-to-date guidelines that tackle Life Cycle Assessment (LCA) modelling of CCU, primarily the latest Together for Sustainability (TfS) guideline promoted by the chemical industry; notably, this approach contrasts with the broader and more holistic discussions prevalent in the scientific community. The goal is to put such guidelines to the test, enhancing their critical evaluation, and assessing their cascading effects throughout the supply chain. The preliminary findings, derived from modelling according to TfS guideline, indicate a carbon footprint reduction when transitioning from fossil-based to Blue Ammonia. Further dive into the details of the guideline showcase that employing system expansion via substitution of Direct Air Capture technology yields mixed outcomes. The impact of such substitution on the results is contingent upon several factors, mainly the region of operation and the subsequent energy mix selection. Alternative modelling approaches can result in no shared incentives between CO<sub>2</sub> producer and user, such as employing system expansion via substitution of Ammonia production without CCU. In such cases, the reported environmental impact reduction applies only to the user of captured CO<sub>2</sub>, with no further credit incentives for Blue Ammonia user. This research not only evaluates the progress made in industry-specific modelling principles for LCA of CCU, but also underscores the considerable room for improvement in existing guidelines.

##### **5.04.B.T-02 Sustainable Transition to a Circular Economy: Modelling Environmental and Socio-Economic Impacts of Regional and National Waste Management Systems in Europe**

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Waste is being produced at a faster rate than any other environmental pollutant and is expected to increase dramatically over coming years. In response, world leaders are increasingly embracing the idea of a circular economy, where materials are reused and regenerated rather than discarded. In this regard, the European Union (EU) has established regulatory targets that mainly prioritize recycling over incineration and landfill. This will inevitably lead to a range of technological developments and new product design requirements to facilitate recycling, reuse and refurbishment. However, rapid changes may pose challenges in adaptation. This could result in the mismanagement of waste, which, in turn, can have detrimental consequences on human health and the environment. Thus, it is imperative that the impacts of waste management systems are assessed to ensure resource circularity while avoiding adverse effects. In addition, the existing targets focus exclusively on municipal waste. It is important to evaluate not only the effectiveness of these targets and whether they appropriately target specific streams and sectors but also the necessity of setting targets for non-municipal fractions. Moreover, the environmental, economic, and social impacts of both the current waste management system and the potential consequences associated with future changes should also be assessed. However, currently, no studies provide a comprehensive and dynamic system-level model based on life cycle thinking that enables the consistent assessment of all waste streams occurring in the EU, addressing the three sustainability pillars, namely environmental, economic and social impacts. This study contributes to an ongoing EU project that provides a flexible modelling framework, that can assess both EU and country-specific waste management systems and is adaptable to changes in framework conditions, technology options, and regulatory focus. The model has the ability to assess different scenarios (e.g. imposing an environmental tax on a material to assess its effectivity, etc.) and produce results regarding recycling rates per waste stream, overall recycling rates, 16 environmental impact categories, internal costs, external costs, and employment. At the conference, the model set-up along with preliminary results will be presented to provide insight into how such frameworks can be leveraged in policymaking to ensure an efficient and sustainable transition towards a circular economy.

#### **5.04.B.T-03 Supporting decision-making on municipal utility fleets decarbonization considering inventories and impact assessment dynamics**

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Due to the dynamic nature of battery electric vehicles (BEVs), assessing their environmental impacts requires dynamic LCA (DLCA) coupled with dynamic life cycle inventory (D-LCI) data and dynamic life cycle impact assessment (D-LCIA) methods.

Unlike the abundance of studies focusing on impacts from passenger/freight transportation, municipal utility fleets (MUFs), however, have rarely been scrutinized. Using MUFs driven in Quebec, Canada as the study object, we incorporate both D-LCI and D-LCIA (focusing on Global Warming Potential (GWP)) to evaluate the potential impacts on electrification of Internal Combustion Engine Vehicle (ICEV) MUFs.

For D-LCI, we prepare dynamic foreground inventories and adopt premise-adjustedecoinvent database as the background dynamic inventory. For D-LCIA, we propose a d-GWP<sub>SSPx</sub> approach by integrating future projected climate scenarios, i.e., calculating d-GWP using the CO<sub>2</sub> concentration under different Shared Socioeconomic Pathways (SSPx, x = 1/2/3/4/5). The DLCA is conducted to compare a conventional ICEV-diesel(-d) with a BEV garbage truck driven in Montreal, Quebec, Canada. For the BEV, two types of battery were tested: the currently commercialized lithium nickel manganese cobalt oxides battery (LIB-NMC622) and a promising lithium-sulfur battery (LSB). We averaged the vehicle lifetime impacts to a functional unit of vehicle-km (vkm). The methodological consistency is ensured. The LSB investigated are expected to be deployed only after 2030, under either SSP1 (taking the Green Road) or SSP2 (Middle of the Road) scenarios. We tested with two Integrated Assessment Models (IAMs), i.e., image SSP2-Base and image SSP2-RCP26.

Preliminary results provided us with unanticipated findings. Both LSB- and NMC-BEV received slightly worse (~9% higher impact score) as compared to ICEV-d in 2030. Overtime, the LSB-BEV improved by 6% and 23% as compared to ICEV-d in 2050 on climate change under image SSP2-Base and SSP2-RCP26, respectively.

The DLCA study showed us that electrification of a garbage truck does not necessarily yield positive climate change consequences, especially when new battery technology readiness level is still low. However, the conclusion is dynamically changing with the evolving foreground and background systems.

#### **5.04.B.T-04 Consequential Life Cycle Assessment of Fuels for Shipping**

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While the maritime transportation sector is responsible for the movement of up to 90% of the world's goods, it is also responsible for 3% of global GHG emissions. The awareness of the effects of these emissions has led to regulations on decreasing emissions from fuel combustion, and changing to low-carbon or carbon-free fuels has been identified as a critical

means of achieving these targets. A variety of alternative fuels for shipping have been identified as promising, although there is no consensus yet on what the future fuel mix looks like.

Life cycle assessments (LCAs) have been used to support decision-making in various sectors, and several life cycle assessments of maritime fuels have been conducted and published in scientific journals, with many of these focused on conventional fuel oils. Despite an increasing representation of alternative maritime fuel LCAs in literature, the existing literature is limited in its inclusion of critical elements and transparency in reporting methodological choices and assumptions. The diversity of fuel types and fuel production pathways, coupled with inconsistent LCAs on these fuels complicate decision-making regarding fuel choice.

In this work, consequential LCA is applied to a range of different maritime fuel scenarios, to demonstrate how to address the existing gaps in literature. The system boundaries include both production and combustion of the fuels. The function unit is chosen as 1 tonne of cargo transported 1 kilometer (1 tkm) to address differences in fuel energy density. Multiple literature sources of data are used to provide a range of potential impacts for several different impact categories.

Additionally, the results of the environmental life cycle assessment scenarios are contextualised with regards to regulatory frameworks and certification schemes for fuels, such as the EU's Renewable Energy Directive (RED) and the the Low Carbon Fuel Standard (LCFS).

It is expected that the outcomes of this study will provide guidance to LCA practitioners and decision-makers for i) how to conduct comprehensive LCAs of alternative maritime fuels, and ii) decision support regarding fuel choice and the expected ranges for the various fuels.

#### **5.04.B.T-05 Lessons Learned From a Practitioner-Driven Project on the Sustainability Assessment of Regional Products**

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Quantitative evidence of the economic, environmental, and social sustainability of consumer products is difficult to find and scientific studies on the evaluation of Protected Designation of Origin (PDO) products are scarce. The one-year DurAOP project aimed at developing a methodological framework for the sustainability evaluation of PDO products and applying it to five Swiss PDO products. The aim of this work is to reflect on the lessons learned from the DurAOP project in terms of methodological development of the sustainability assessment methods, project co-creation, and communication challenges. The project was carried out in four main steps: First, the development of the methodological framework, second, the data collection, third, the results generation and fourth, the presentation and interpretation of the results. Several methodological challenges arise in the first three steps of the project, namely: (1) defining quantitative indicators able to reflect trade-offs for the social and economic dimension, (2) reflecting the specificities of regional production, (3) ensuring the quality and comprehensiveness of the collected data and the representativity of default data, and (4) allocating the impacts to the analysed product, or more generally going from a company/farm level to a product level. In addition, the communication of disaggregated results for several indicators across the three sustainability dimensions has proven difficult. We tried to tackle the methodological challenges by keeping the indicators of the social and economic dimension simple, ensuring a good overlap with the available data, and defining new indicators reflecting the regional character of the products' value chains. We further visited all the producers and conducted plausibility checks to increase the confidence in the collected data. Finally, allocation was avoided as much as possible by modelling only the processes relevant for the considered products. The combination of in-person meetings and streamlined results presentations aimed to facilitate the communication of the projects' outcome. In addition, a transparent communication of the study's strengths and limitations contributed to the confidence in the presented outcome. Overall, the DurAOP project allowed the development of a first methodological framework for the sustainability evaluation of PDO products and the evaluation of the sustainability of five Swiss PDO products.

#### **5.04.P LCA in Policy, Decision-Making and Communication to Support the Transition Towards Sustainable Consumption**

##### **5.04.P-Tu475 A theoretically based approach to reconcile different types of GHG-accounting**

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Greenhouse gas (GHG) emission accounting plays a pivotal role in guiding climate actions and policy decisions. In the contemporary landscape, diverse objects, including countries, cities, regions, organizations, products, projects, and individuals, engage in GHG-emission accounting. The resulting accounts underpin critical decisions related to climate target comparisons, policy instruments, investments, and consumption choices. While global initiatives such as the GHG-Protocol and the International Standardization Organisation (ISO) have endeavored to standardize GHG accounting, these standards emerge from complex negotiation processes driven by diverse interests.

This study critically examines existing GHG accounting standards, revealing inherent conceptual challenges arising from the absence of a holistic, theoretically grounded foundation. The lack of unified definitions for central terms, inadequate

consideration of interdependencies between GHG accounts, variations in handling temporal dimensions of accounting, and the failure to distinguish between different forms of double counting contribute to the fragmentation of current standards.

In response to these challenges, our work presents a theoretically based approach aimed at assessing the foundational principles of GHG emission accounting. By identifying conceptual challenges in existing standards, we propose solutions to facilitate the reconciliation of diverse GHG-accounting types. This research contributes to a comprehensive understanding of current GHG-emission practices, shedding light on the necessary steps to transform GHG accounting into a robust asset for addressing climate change.

The outcomes of this research hold significant implications for policymakers, practitioners, and researchers, offering insights that can inform the development of more coherent and effective GHG accounting standards. Ultimately, this work contributes to advancing our collective understanding of how GHG emissions are accounted for across different objects and lays the groundwork for enhancing the role of GHG accounting in the global effort to combat climate change.

#### **5.04.P-Tu476 Alternative pathway for evaluating the environmental impacts of industrial plants with publicly collected data**

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Assessment of industrial plants and technologies at the level of only one environmental indicator, such as carbon footprint, can cause an insufficient view and negative environmental impacts at the local level, e.g. increased acidification, eutrophication, a decrease of mineral resources, etc.

It is necessary to update and expand the standard approach for evaluating new low-emission technologies and systems to include other environmental impacts to limit or avoid these negative aspect, thereby limit the potential adverse effect of shifting one environmental problem to another.

The paper will present a structure of designed alternative approaches for evaluating the environmental impacts of industrial plants in the Czech Republic, primarily developed for the public sector. Environmental assessment and indicators are determined using the life cycle assessment method (LCA) with the implementation of the European methodology, PEF 3.1, i.e., Product Environmental Footprint. The PEF 3.1

The developed methodology defines and establishes a set of environmental impact indicators that can be used for a comprehensive and transparent assessment of industrial operations at the level of the source, sector and even regions/the whole of the Czech Republic using data collected today by the public sector for statistical purposes, e.g. IRZ or E-PRTR, the database of the Statistical Office of the Czech Republic etc.

The alternative pathway is built on the structure defined by the European Investment Bank for the evaluation of new projects at the level of the value of the so-called carbon footprint. It expands it by other environmental indicators and the use or treatment of solid waste as alternative secondary raw materials for producing materials or products with the implementation of the so-called industrial symbiosis approaches.

Finally, the paper will show the results of using the proposed approach and environmental indicators to assess selected heavy industrial plants.

#### **5.04.P-Tu477 A systematic review of the life cycle assessment of clothing**

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According to McKinsey & Company (2020), the fashion industry emitted about 2.1 billion tons of GHG in 2018, and there is an urgent need to address this issue. In addition, global clothing sales nearly doubled from 50 billion to 100 billion items from 2000 to 2015 due to the growth of the world population and the growing middle class; by 2050, it is estimated to be more than three times the current level. In addition, the exit disposal phase has all kinds of environmental impacts, with 73% of used garments going to landfill or incinerated at the exit of the global garment material flow, according to the Ellen MacArthur Foundation (2017). In this study, we surveyed the literature and attempted to identify representative clothing and their environmental impacts from fiber materials in the processes of spinning, fabric manufacturing, dyeing, and sewing. As a result, for cotton fiber, many data were reported up to cotton cultivation. However, it was found that there are only a limited number of reports that include the process of separating the cotton fiber from the seeds, known as ginning, after cultivation. GHG emissions per kg of cotton fiber ranged from 1.33-4.43 kg-CO<sub>2</sub>e, with Cotton Inc.'s 1.33 kg-CO<sub>2</sub>e being the most frequently cited. As reported by Prabod Munasinghe et al. (2021) and Baydar et al. (2015), the environmental impact of the wet process, including the dyeing process, was found to account for a high proportion of the clothing manufacturing life cycle. For jeans, a

typical garment, we found that the report by Levi's is often cited and treated as a standard. Among those surveyed in this study, the range varied from 5.53-68.5 kg-CO<sub>2</sub>e per pair of jeans manufactured. Factors contributing to this included the use of utilities and steam in factories, and the country and method of manufacture.

#### **5.04.P-Tu478 How Does Global Palm Oil Consumption Impact Indonesia's Biodiversity and Ecosystem Services?**

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Indonesia's economy heavily depends on palm oil production but faces significant environmental challenges, particularly regarding biodiversity and ecosystem service degradation. This study aims to analyze the tele-connected impacts of global palm oil consumption on Indonesia's biodiversity and ecosystem services through a sub-national landscape-scale analysis. We will first integrate the latest satellite remotely sensed earth observation datasets (e.g., high-resolution maps of oil palm plantations) with state-of-the-art landscape-scale biodiversity and ecosystem service models for impact assessment. Then, we will calculate the impacts of oil palm cultivation per unit area/unit production at the district scale. The above two steps will allow us to identify specific and overlapping environmental hotspots of oil palm cultivation with high spatial explicitness and, therefore, address the so-called double materiality of the oil palm industry (dependence and impacts on nature). Finally, we will link district-scale export supply chains with country-scale multi-regional input-output (MRIO) tables to track the tele-connected impacts from cultivation to consumption. Preliminary results show a direct link between global palm oil demand and the degradation of biodiversity, carbon sequestration, water purification, and intact forest landscapes in Indonesia. Our detailed results aim to provide policymakers and stakeholders with a thorough understanding of these interlinked impacts, which will be essential for developing strategies to mitigate the environmental impacts of palm oil production. This, in turn, can influence local and international policy decisions towards more sustainable consumption patterns. Furthermore, the presented approach can be extended to assess other commodities and sectors with high spatial details.

#### **5.04.P-Tu479 Impact assessment of relocating and improving the recycling of scrap metal from end-of-life vehicles and waste electrical and electronic equipment in Wallonia, using the life cycle assessment tool.**

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In response to the rising production of vehicles and waste electrical and electronic equipment (WEEE) amid decreasing resource availability, the Wallonia region in Belgium has initiated "Reverse Metallurgy +" projects. These projects focus on circular economy practices (metal, battery, and mineral reuse, upscaling, and recycling) and low-carbon industry development to meet CO<sub>2</sub> emission reduction targets in metallurgy and construction.

Key challenges include relocating metal scrap treatment to Europe for a sustainable local recycling industry and enhancing recycling processes for high-purity, high-value metal streams. The aim is to optimize metallic material recycling for more circular metal production in Europe, assessed using the Life Cycle Assessment (LCA) tool.

Several projects from "Reverse Metallurgy +" are part of this approach for circularity in the metal supply chain. These include "CISTEMEEC" (in the field of light electric mobility with Li-ion, copper and rare-earth batteries); "ECWALI" (for high-purity sorting of aluminum alloys and stainless steels); "PYROTECNIC" (development of a pyrometallurgy industry and intelligent packaging techniques for the circular economy) and "REMADE" (valorizing recycled metals in very high value-added products by developing various production and utilization channels for metal powders, in particular for additive manufacturing).

In the "CISTEEMIC" and "ECWALI" projects, the aim is to estimate the impacts of a Belgium recycling site which uses innovative technologies and to compare these values with those in the literature in order to conclude on the various benefits. Once this recycling process has been estimated, it will then be possible to estimate the benefits of additive manufacturing in the "ECWALI" project.

There are no results yet, as we are still in the data-gathering phase. The impact of metal recycling, based on the literature, should be calculated by the time of the conference. The comparison with the impacts of the projects mentioned will depend on the availability of company data, which is also linked to their ability to isolate metal flows during recycling and production.

Ultimately, these projects seek to estimate the benefits of high-quality metal recycling in Europe, aiding companies in strategic decision-making for technology development to address climate emergencies and market demands.

#### **5.04.P-Tu480 Using Life Cycle Assessment (LCA) to foster the renaissance of the European photovoltaic (PV) manufacturing industry**

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Solar energy has become a key player in the transition towards a cleaner energy system and its deployment is essential to achieve the climate targets set by the Green Deal.

The EU has ambitious plans to promote and accelerate the use of solar energy. At the moment, PV manufacturing industry is mainly retained in China. Diversifying supply chains and delivering more efficient and sustainable products is essential to ensure a secure and successful energy transition in Europe. However, the abrupt drop in prices of PV modules puts under imminent risk the renaissance of the European PV manufacturing industry. To tackle this critical situation, LCA could be a useful tool to show the environmental advantages of pushing for a resilient solar industry in Europe. Also, it can be used to highlight the main challenges that need to be addressed at each phase of the solar panel life.

To support the development of new photovoltaic technologies in Europe, a cradle-to-gate LCA is performed to study the hotspots of a potential mainstream technology: silicon heterojunction tunnel – interdigitated back contact technology and assist the eco-design phase. Hereafter, it will be extended to a cradle-to-grave LCA, to analyze the impacts during the use phase and the end-of-life. Different background scenarios are considered to compare the impact of importing Chinese panels with EU manufactured ones.

To perform the described analysis, Product Environmental Footprint Category Rules (PEFCR) for PV electricity are followed, and the E.F 3.1 impact assessment method is used. Primary data for the SHJ-IBC is collected from technology manufacturers and secondary data is mainly obtained from the inventories of the International Energy Agency. Ecoinvent 3.9.1 is used as database and the modellings are done in the software Simapro.

Results will show to what extent emerging PV technologies present an environmental advantage compare to imported modules. Also, the analysis of how the manufacturing location and technical characteristics make a difference to reduce the environmental footprint could make a shift to stop focusing only on the economic dimension and put more weight on sustainability aspects.

Ultimately, on this critical moment when European solar industry is at risk of disappearing, LCA can be used as decision-making tool to transform solar module panels “made in Europe” into a real sustainable technology that can lead the energy transition towards a cleaner and dematerialized society.

#### **5.04.P-Tu481 Improving the Availability of High-Quality Environmental Impact Data for Key Food Items Through HESTIA**

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The availability of high-quality, granular, comparable, and representative environmental impact data for food items provides a number of important opportunities. However, many existing databases fall short on at least one of these requirements. We have been working to improve the data in this area with a focus on UK-consumed food. The work has involved: improving the quality of data available for high-impact food commodities; understanding the impacts of multi-ingredient food items and how their variability is distributed; and enhancing a model of the UK food and drink sector GHG footprint. The aim of the work is to build a comprehensive understanding of how UK consumption impacts the environment, how those impacts vary, and how uncertainty affects their variability, with implications for eco-labelling.

To obtain consistent, comparable data for on-farm impacts, the HESTIA platform was used to upload, recalculate, and store activity data from farm surveys and LCA studies. To analyse multi-ingredient food item impacts, information was obtained through the foodDB database, and then linked to the UK’s National Diet and Nutrition Survey and to HESTIA data. Variability of impacts was analysed through Monte Carlo simulations. The outputs have been used within WRAP’s GHG model of the UK food system to provide a holistic understanding of the impact of the production and consumption of food and drinks.

Our results show the power of using consistent data infrastructure to recalculate impact estimates in an automated way. It has the potential to transform the food data system, enabling transparency and comparability of data to aid decision-making. Monte Carlo analysis coupled with such data can help policy makers understand requirements for on-pack environmental impact information, and provide hotspot analysis to focus businesses’ efforts most impactfully.

We plan to further develop this work by: embracing large datasets to accelerate HESTIA’s data coverage; supporting integration of HESTIA infrastructure in businesses; scrutinising existing eco-label methodologies; and providing recommendations for how eco-labels can be developed with integrity. This is an opportunity to improve the availability of high-quality, granular, and representative data for food system transformation. Future partnerships between us, industry and academia can help make food LCA data available in a consistent and transparent manner to substantially reduce environmental impacts.

#### **5.04.P-Tu482 Methodological Framework for Life Cycle Assessment of Hydrogen Production Technology via Non-Thermal-Plasma Methane Cracking**

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Hydrogen (H<sub>2</sub>) will play a key role in the economy sustainability challenge and energy transition.

In this frame Coldspark is a European project exploring the production of H<sub>2</sub> via non-thermal-plasma (NTP) methane cracking, which technology represents an innovative solution to produce high-pure H<sub>2</sub> and solid carbon at low temperatures and ambient pressure without the use of any catalyst and with no direct carbon dioxide (CO<sub>2</sub>) emission.

The environmental sustainability evaluation of the technologies through the life cycle assessment (LCA) methodology represents a crucial step in early projects to help the researchers in selecting the most optimal technical solution. Therefore, a preliminary LCA of the process is a fundamental tool to assist the researcher in the process development and assure the technology alignment with the environmental sustainability criteria.

The LCA methodology supported by the ISO 1404/44:2006, involves a comprehensive evaluation of the potential environmental impacts of the product system through the life cycle stages, its development in the European context is represented by the ILCD handbook and by the definition of the European Environmental Footprint method (EF). Despite this guidance, the existing LCA studies in the H<sub>2</sub> sector have incorporated various methodological choices that significantly impact their findings and integrations. In this vein, different harmonization efforts were made through the development of more specific guidelines, such as the LCA protocol for fuel cell and H<sub>2</sub> energy systems elaborated by FC-Hy Guide. Additionally, specific greenhouse gas emission (GHG) calculation methodologies are defined and in development respectively for the renewable and low carbon H<sub>2</sub> classification in the European legislation frame. In this research the authors focused on the Hydrogen LCA methodological framework identifying common methodological aspects which will constitute the basement of the NTP methane cracking LCA model. The selected LCA parameters according to the unified recommendations and the technology specifics are: 1) Functional unit: 1 kg H<sub>2</sub> and 1 MJ H<sub>2</sub>; 2) System boundaries: complete life cycle of H<sub>2</sub>, covering production to disposal while excluding the use phase; 3) Multifunctional approach: system expansion.

#### **5.04.P-Tu483 PEF-wise methodology for bio-based fertilisers: A first normative proposal, critical analysis, and further perspectives**

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Bio-based fertilisers (BBFs) promotion is a key element in the Farm-to-fork strategy and European Green Deal to reduce the dependency on mineral fertilisers. Mechanisms to promote the most environmentally effective products will maximise environmental benefits and prevent rebound effects. Life Cycle Assessment (LCA) is the most suitable tool. Nonetheless, one of the main criticisms is the divergent methodological decisions that hinder its reliability and comparability. The European Commission has developed the Product Environmental Footprint (PEF) initiative which aims to create a harmonised LCA method for products and provide a common way of measuring different sectors and products through the Product Category Rules (PCR). This work presents the first proposal for a PEF-wise PCR of BBFs. For its development, a review of the scientific literature, standards and guidances was done. Then, the methodologies were adapted to the PEF framework. Moreover, other normative frameworks, such as Environmental Product Declarations and specific PCRs were consulted. When developing a PCR, decisions are needed on the definition of the Functional Unit (FU), reference flow, system boundaries, allocation methods, and further specification of the use of the Circular Footprint Formula. This first proposal assumes that BBFs and fertilisers are intermediate products of agricultural systems and are embedded in other supply chains. Therefore, to propose a PEF-wise PCR from the raw material/biomass collection to the distribution of BBF at the biorefinery gate (Cradle-to-Gate). The application stage shall be included in other products' PCRs. Due to major differences in the nutrient content of the BBFs and their fertilization efficiency, no common FU could be derived. Thus, only the reference flow of the BBF (i.e., 1 kg) shall be given. However, this approach is acknowledged to be limited to the final aim of PEF which is the comparability of fertilizer products and guiding decision-making. Therefore, it is proposed to use complementary FUs and extend system boundaries to include the application stage as additional environmental information (as defined in PEF). Nonetheless, there are technical limitations, such as the variability of the application stage and agronomic performance indicators. Finally, a critical assessment of this normative adaptation reveals some constraints of the PEF framework in creating a suitable and useful mechanism for the emerging sector of the BBFs.

#### **5.04.P-Tu484 Life cycle assessment framework for evaluating the environmental performance of refractories in the steel industry: the choice of magnesia-carbon bricks for steel ladle lining as a case study**

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Refractories are materials that can withstand at very high temperatures and are essential for any high-temperature operations across various industries. With no surprise, the iron and steel industry is the largest end user of refractories, with around 70% of the total market share. Few life cycle assessment (LCA) studies were found in the literature mainly focusing on the production of refractories or refractory raw materials, ignoring their use and end-of-life scenarios. Inconsistency in system boundaries, lack of data, multifunctionality, and uncertainty were identified as major challenges in conducting LCA, comparing different studies, and communicating LCA results with stakeholders. Therefore, methodological guidelines are necessary to address these challenges and communicate LCA results for decision support. In this study, a comprehensive LCA framework is proposed to assess the environmental impact of refractories within the steel industry and the utilization of magnesia-carbon (MC) bricks in the steel ladle lining is specifically examined as a case study. The investigation involves a cradle-to-grave LCA on MC bricks sourced from three alternative suppliers situated in China and Europe. Allocation and



system expansion approaches are applied to deal with co-products and account the benefits of recycling. Inventory data associated with the use phase and end-of-life management of refractories were collected from Tata Steel Netherlands. However, it was not possible to compile refractory production data from suppliers due to confidentiality constraints as inventory data often equivalent to their product recipe. Innovative techniques are developed to retrieve crucial inventory data from product data sheets received from suppliers. Preliminary cradle-to-gate LCA results highlight electricity as a major environmental hotspot, prompting the exploration of different scenarios, including variations in the electricity source. Acknowledging the inherent uncertainty in inventory data, Monte Carlo simulations are planned to be implemented at the inventory level to illustrate the distribution of LCA results. Furthermore, sensitivity analysis will be conducted to test the influences of significant uncertain parameters on results. The primary objective of this study is to provide a comprehensive LCA framework for refractories to enhance methodological practices and communicate LCA results for decision support in the steel industry when choosing refractories.

#### **5.04.P-Tu485 Comparative Life Cycle Assessment of Combined Ethylene and Acetic Acid Production by Oxidative Dehydrogenation of Ethane**

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Steam cracking is a central process in the petrochemical industry for the production of olefins such as ethylene and propylene. In this process, hydrocarbons are thermally cracked into short-chain hydrocarbons at high temperatures and short residence times with the addition of steam. Despite process optimization, the steam cracking process is not climate-friendly due to high process-related greenhouse gas emissions, especially from underfired reactor heating to achieve high process temperatures.

An alternative process is the oxidative dehydrogenation of ethane (ODHE). This is a catalytic, exothermic process that allows direct dehydrogenation of ethane with the addition of oxygen. The process is carried out at much lower temperatures and does not require underfired reactor heating. This can save a large amount of direct CO<sub>2</sub> emissions in the production of ethylene. Another by-product of the catalytic reaction and an additional value product of the overall process is acetic acid. Linde GmbH developed a commercial-scale process based on the ODHE, the EDHOX™ process.

In the context of this work, the potential of the novel process for the ODHE to avoid climate-relevant emissions in the production of basic chemicals will be investigated in a comparative life cycle assessment (LCA).

The EDHOX™ process is compared to a conventional steam cracking process for the production of ethylene. To solve the multifunctionality problem of the EDHOX™ process, the steam cracking process is extended by means of system expansion with a conventional production process for acetic acid. In addition, further potentials are being investigated for making both the EDHOX™ process and the existing processes more sustainable, for example through energy integration or successive electrification of subprocesses. Thus, future decarbonization strategies in chemical industrial processes are investigated and included in the comparative LCA.

#### **5.04.P-Tu486 Challenges and Insights in the LCA of Perovskite PV at Low TRL**

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The European Union has positioned itself at the forefront of the global effort to transition from fossil fuels to renewable energy sources and curb greenhouse gas emissions, with a specific focus on achieving climate targets by 2030. Notably, the photovoltaic market for electricity generation has witnessed substantial growth in recent years, driven by the imperative to address climate change.

Perovskite photovoltaics (PV) has emerged as a notable subject of interest among researchers in the photovoltaics field. In laboratory settings, perovskite PV exhibits distinct advantages, including reduced thickness, cost-effectiveness in production, and higher efficiency compared to other photovoltaic technologies. Researchers assert that the efficiency of Perovskite PV can reach up to 25%, with stability demonstrated over several thousand hours. The lightweight nature of perovskite PV has opened up diverse applications in aerospace, automotive, and other industries.

Recognizing the environmental implications of this technology, conducting a life cycle assessment (LCA) is imperative. Unlike many previous LCA studies that focused on specific stages like production or end-of-life, this study aims to conduct a comprehensive attributional LCA of perovskite PV, covering its entire life cycle from cradle to grave. Key obstacles hindering the extensive adoption of this study involve issues with data quality, inadequate inventory data, absence of a dedicated LCA methodology, and significant scale-up issues.

In this LCA study, Primary data have been gathered from project partners, supplemented by secondary data from Ecoinvent v3.9.1. The LCA study is done according to the ISO 14040/44 standards and also followed by PEFCE and International Energy Agency(IEA) Guidelines and harmonized with other PV projects across Europe to ensure methodological rigor. Additionally, sensitivity and uncertainty analyses are incorporated to assess the reliability of the data and address uncertainties. This final study contributes in understanding how the Perovskite PV impact varies in different environmental impact categories throughout their life cycle.

#### **5.04.P-Tu487 Environmental assessment of producing fossil and bio-based polyurethane foams: a review**

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The global polyurethane (PU) market projections indicate growth will continue up to 27.61 million tons in 2026. Their environmental impact is a matter of significant concern as so far, the production of PUF is essentially dependent on fossil materials, namely isocyanates and polyols. European Green Deal aimed at reducing greenhouse gas (GHG) emissions and decoupling economic growth from resource use, therefore, urgent action is needed to find alternative raw materials and optimize manufacturing processes. In line with that, the use of renewable polyols is already a reality in the PUF industry, namely vegetable oils, recycled polyols, and CO<sub>2</sub>-based polyols. However, this only contributes in part to the total polyol used in some formulations in various industrial-scale applications. Even though there are other potential renewable polyol counterparts such as lignin, as well as alternative synthetic routes to produce PUF including variations of the so-called via Non-Isocyanate Polyurethane (NIPU), there are still several limitations compared to the conventional manufacturing processes involving petroleum-derived raw materials. For that reason, most of these alternatives are not yet available. Furthermore, the possible benefits of these potentially greener alternatives need to be compared with the environmental performance of well-established conventional processes. Yet, only a few life cycle assessment (LCA) studies exist on PUF. Moreover, a comparison of impact results between different LCA studies is only possible whether the methodological choices and assumptions of each study are equivalent.

This review work presents the results from a systematic literature analysis regarding LCA studies on PUF produced using a variety of raw materials, classified as four different feedstocks in this work. This timely work identifies the main trends in methodological choices, including functional unit, system boundaries, multifunctionality, impact assessment methods, and sensitivity analysis, and enables the understanding of their effect on the environmental sustainability of PUF, namely in the most evaluated impact category- the climate change (CC). A wide range of CC variation was observed, ranging from 2.95 kgCO<sub>2</sub>eq/FU for a fossil-based to 7.67 kgCO<sub>2</sub>eq/FU for a recovered fossil polyol. The main issues for this high variability are addressed, highlighting the need for further harmonization and consistency in data collection and methodological choices used in LCA studies.

#### **5.04.P-Tu488 Environmental impacts of alternative dross utilization from primary and secondary aluminium production**

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Dross in context of the aluminium production is a mixture of both metallic and non-metallic materials which forms mainly due to the rapid oxidation of molten aluminium upon contact with air. The loss of aluminium and the formation of aluminium oxide is unwanted from both economic and technological perspective. The formation of dross has also significant environmental impact and wrongly attended dross poses substantial threat to surrounding biosphere. Around 5 million tons of aluminium dross is produced yearly. Despite noticeable progress over past decades in both the minimalization of the dross production as well as the further utilization, significant amounts of mainly black dross are still being deposited.

The goal of the presentation is to identify and assess of potential environmental impacts or benefits of novel pathways and improvements of dross utilization developed in ABTOMAT project. In addition, the effects of the implementation of the proposed solutions on the environmental footprint of aluminum will be analyzed.

Environmental impacts and indicators are evaluated using the Life cycle Assessment (LCA) approach based on Product Environmental Footprint (PEF 3.1) methodology. The analyses is focused primarily on the operation phase. The evaluation is performed by Expert for LCA software with specific databases (Sphera – 2023, Ecoinvent 3.9, ILCD, EF).

#### **5.04.P-Tu489 Life Cycle Assessment to Support Bioenergy Integration in Tissue Paper Production**

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The consumption of tissue products such as toilet paper, napkins and kitchen towels is increasing globally, leading to increased environmental impacts. The integration of pulp and tissue paper production in the same industrial site arises as a strategy to decrease these impacts. This integration allows the transfer of slush pulp (in water suspension) to the tissue mill displacing market pulp (dried and sold in bales), avoiding pulp transport and drying. Furthermore, it enables bioenergy transfer from the pulp mill to the tissue mill replacing energy produced from fossil fuels. However, the environmental performance associated with this strategy remains unclear. In this study, life cycle assessment was applied to support decision-making regarding the implementation of bioenergy integration in a typical industrial site in Portugal producing eucalyptus pulp and tissue paper.

Two scenarios differing in the sources of steam and electricity consumed in the tissue mill were analysed. In the scenario without bioenergy integration, steam is produced on-site from natural gas and electricity comes from the national grid. In the

scenario with bioenergy integration, steam and 33% of the electricity are produced on-site in the biomass boiler of the pulp mill, and the remaining electricity comes from the national grid. A cradle-to-gate perspective was adopted using primary data based on real operations. The ReCiPe 2016 impact assessment method was applied as default and the Environmental Footprint (EF) method was used in a sensitivity analysis.

The results show that the integration of bioenergy in tissue production leads to a decrease in the environmental impacts of up to 20% with the ReCiPe method except for the categories of marine eutrophication and mineral resource scarcity. Marine eutrophication increases by 22%, whereas mineral resource scarcity remains similar. The application of the EF method results in similar trends, except in the case of marine eutrophication which has similar results in both scenarios.

In conclusion, bioenergy integration in tissue production contributes to a better environmental performance of tissue products, but additional measures are required to achieve improvements on marine eutrophication and mineral resource scarcity. These results are paramount to support decision-making concerning processual changes in the pulp and paper industry and also for authorities in charge of defining environmental policies, incentives and tax regulations.

#### **5.04.P-Tu490 Lowering Laundry and Dishwashing Environmental Impacts in Europe from a LCA perspective**

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Climate change exerts a significant and far-reaching influence on the global environment, economies and society. It is imperative for all stakeholders, including manufacturers and consumers, to proactively engage in understanding and mitigating their environmental impact. The findings presented in this study reveal the outcomes of three Life Cycle Assessments carried out by Procter & Gamble in European markets, aligning with ISO standards, to comprehensively evaluate the environmental footprints of laundry, automatic dishwashing, and hand dishwashing detergents. The primary goals were to pinpoint key impact areas and compare prevailing consumer practices with more sustainable product usage.

Across the three product categories, the use phase emerged as the most significant contributor to the carbon footprint, constituting 60% for laundry detergents, 73% for automatic dishwasher detergents, and 90% for hand dishwashing detergents. Moreover, the use phase dominated in various other impact categories, including water scarcity, fine particulate matter, and fossil resource scarcity. This dominance is intricately tied to the energy required for heating water during the washing process, influenced by the energy composition of each country- be it fossil fuels, renewable sources, or nuclear power. As a consequence, the carbon footprint varied across different markets, with lower emissions observed in nations boasting a higher proportion of nuclear energy (e.g., France) or renewable energy (e.g., Sweden). Nevertheless, future scenarios for laundry detergents suggest that, despite the anticipated greening of the electricity grid by 2030, the use phase will remain the primary driver of environmental impact for these product.

The scenarios of sustainable product usage underscored that reducing energy consumption by employing lower wash temperatures in laundry and hand dishwashing or adopting energy-efficient short cycles in dishwashers, holds the most significant potential for carbon footprint reduction (16% for laundry, 60% for hand dishwash, and 33% for automatic dishwash). These study results emphasize the necessity for a holistic approach in evaluating the environmental impact of detergent products and guiding informed actions and business decisions. This involves considering various aspects, including sourcing, formulations, packaging, and promoting sustainable usage through product innovation and consumer education to foster environmentally conscious habits.

#### **5.04.P-Tu491 What Are We Missing in the Climate Crisis? A New Role for Healthcare Systems**

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Healthcare systems (HSs) play a crucial role in providing support and care to populations worldwide, yet they demand a considerable amount of energy to fulfill their responsibilities. Specifically, HSs directly contribute to global warming and environmental footprint, accounting for up to 5% of global net carbon emissions. This impact can be classified into three scopes based on the source type: scope 1 emissions originate directly from healthcare facilities and vehicles, scope 2 emissions result from purchased energy sources, and scope 3 emissions encompass all other indirect emissions triggered by the healthcare supply chain. This evidence highlights a paradox where HSs while maintaining their role in health protection, may increase risks to collective health. In this scenario, health prevention is at risk of failure, and achieving the zero-carbon dioxide (CO<sub>2</sub>) emissions targets set by the Paris Agreement in 2015 by 2050 seems unlikely without limiting the impact of HSs, alongside other industries, on climate change. This study aims to reconsider the role of HSs and propose a shift during health prevention. We conducted a rapid scoping review, searching all PubMed databases and selecting only peer-reviewed quantitative studies that delve into direct and indirect CO<sub>2</sub> emissions attributed to HSs. To date, the available data is limited, though a slight increase in recent years offers some hope. Most emissions appear to stem from the supply chain on which HSs rely to perform their tasks. In addition, the analysis of the impacts that HSs have on the carbon footprint varies among countries. Less economically developed countries are underrepresented, and even in economically developed countries, most studies come from English-speaking countries. Our literature search underscores the need for a more concerted effort in investigating healthcare sector emissions. Furthermore, given the global nature of the issue, shared guidelines should be implemented among

HSs of different countries to collectively reduce emissions and reorganize procedures for less impactful actions while maintaining high-quality care. As healthcare sector professionals, we advocate for promoting more sustainable ways of working and emphasize that health workers and policymakers cannot ignore the inherent environmental implications of HSs activity concerning climate change and related health issues.

#### **5.04.P-Tu492 Which LCA methodology for hydrogen-based policies?**

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EU policies rely more and more on life cycle assessments for the green transition. For instance, the carbon border adjustment mechanism (CBAM) will rely on LCAs to determine the taxation for goods entering the EU market. Another example is the renewable energy directive, which provides an LCA methodology to determine whether a fuel can be considered renewable or low-carbon. Hydrogen is interested by both policies, since it can be a renewable or low-carbon fuel, and it will be traded under the CBAM rules. Depending on the LCA methodology, the carbon footprint of hydrogen can vary significantly. For instance, depending on the multifunctionality approach adopted the carbon footprint of hydrogen produced via chlor-alkali electrolysis can be as low as 1 kg CO<sub>2</sub>e/kg H<sub>2</sub> or as high as 11 kg CO<sub>2</sub>e/kg H<sub>2</sub>. This could create inefficiencies and market distortions. Another potential source of discrepancies is the way the carbon intensity of the electricity input for hydrogen production is determined: e.g., if the additionality criterion is considered when renewable electricity is used, or if the production- or consumption-based mix are considered when electricity is sourced from the grid.

Our work explores how the carbon footprint of hydrogen can vary depending on the approaches adopted in the LCA methodologies used by the EU policies. The goal is to understand which approaches should be adopted in LCA-based policies to maximise their objectives: i.e., to foster the green transition and minimize the environmental impact generated by the hydrogen consumed in the EU. Preliminary results suggest that potential consequences outside the boundary of the system investigated should be included in the methodology to avoid unintended outcomes. For instance, if the additionality principle is not included in the LCA methodology, hydrogen produced from non-additional renewable hydrogen could not lead to a net reduction of the overall emissions. This because the renewable electricity would be simply taken away from an existing user. The same applies to the consumption vs production mix: a consumption-based mix should be considered to actually reflect the carbon intensity of the electricity consumed for the hydrogen production. Overall, more research is needed to understand the best approaches to adopt in LCA methodologies for policy purposes.

#### **5.04.P-Tu493 Consequential LCA of the mineral fertiliser consumption patterns in Europe for policy making.**

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Fertilisers have guaranteed food security and human demographic growth in the last decades. Nonetheless, agriculture systems have been pointed out as one of the most relevant in the alteration of the nitrogen (N) and phosphorous (P) cycles promoting several environmental threats for humanity such as climate change and water body eutrophication. The European Union has deployed a range of policies such as the Farm-to-Fork strategy and the European Green Deal to mitigate their use by at least 20%. To assess the environmental changes in the demand, consequential life cycle assessment has been pointed out as one of the most suitable tools. However, current databases use consumption average mixes and do not resolve properly the multifunctionality of compound fertilisers. On the contrary, consequential LCA uses marginal suppliers' mixes. That means the identification of the technologies or products that could be affected by a change in demand. In this case, the identification of the fertilisers that could be affected by the reduction of N, P and K demand.

This analysis aims to assess the environmental performance of mineral fertiliser consumption patterns in Europe and the consequences of the potential changes in the as consequence of the policy mechanisms. For that purpose, in this study, different algorithms and linear models have been applied to the database of consumption patterns of the International Fertiliser Association. To improve the robustness of the results a sensitivity analysis has been done considering the time-series length. As a result, the marginal mixes of the 3 nutrients (NPK) sectors were obtained. The inventories included the whole life cycle of the mineral fertilisers. For that, the identified inventories of the marginal mixes were coupled with the different emissions factors to model fertiliser-induced impacts in the application. These factors include the NH<sub>3</sub> volatilisation factors from the EEA, the direct and indirect production of N<sub>2</sub>O from the IPCC as well as other factors for the leakage to groundwater. The inventories were modelled with Brightway 2.5 and Ecoinvent database 3.9 and uncertainty was included. The results allowed the ranking of countries where the political measures can have more rebound impacts on the environment in fossil and mineral resource depletion, climate change, and eutrophication categories as well as correlation with national economic statistics.

#### **5.04.P-Tu494 The impact of implementing strategies in the upstream supply chain on the environmental footprint of a pharmaceutical**

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Greenhouse gas emissions from pharmaceutical production contribute significantly (e.g., 20 % in England) to the total healthcare emissions. As pharmaceutical supply chains are complex systems many activities that contribute to the

environmental impact of pharmaceuticals are outside the direct control of the company and are therefore scope 3 emissions. Hence, it is important to manage these activities to reduce the environmental impact. In this study, the life cycle stages and parameters contributing to the carbon footprint of a pharmaceutical are identified. Additionally, the benefits of implementing environmentally friendly strategies in the upstream supply chain of the pharmaceutical are analyzed. To determine the environmental impact, a life cycle assessment was performed in accordance with the ISO 14044 standard. The IPCC GWP 100 method is used to determine the carbon footprint of the drug product, expressing the impact per blister pack (60 x 500 mg tablets). A cradle-to-grave analysis was executed, considering all life cycle stages from raw material extraction up to end-of-life. To analyze different supply chain strategies, scenarios were developed in which both the solvent procurement strategy and the electricity mix were altered and compared with the reference (current situation). Considering the impact of one blister pack, it appeared that the production of the active pharmaceutical ingredient (API) had the highest contribution (94 %) to the carbon footprint. Zooming in on this production, results show that the highest share of impact can be assigned to the production of the intermediates of the API and to the solvents used in the further production of the API. Comparing the reference with a worst-case scenario, i.e., assuming electricity sources and solvents with a high carbon footprint (due to origin) used for the intermediates and API production, resulted in an increase of carbon footprint with 32 %. However, implementing a best-case solvent procurement strategy and green electricity mix resulted in a decrease of the carbon footprint with 16 %. Therefore, it could be concluded that implementing a best-case solvent procurement with solvents originating from production processes or countries that result in a lower carbon footprint of the solvent and the implementation of a green electricity mix in the production of intermediates (upstream supply chain) and APIs could significantly reduce the total carbon footprint of a pharmaceutical.

#### **5.04.P-Tu495 Organizational climate impact accounting – moving from blame distribution to towards accounting for action**

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In order to reach the Paris Agreements climate target, urgent reductions of GHG emissions are needed. Therefore, there is no time for suboptimized reduction initiatives, and a need for evidence-based decision support. This paper explores how consequential LCA can provide this evidence-based decision support to organizations in order to make decisions that matter. The paper proposes a shift from traditional climate accounting to climate *impact* accounting, in order to quantify the potential impacts of decisions and assess the system wide impacts of a potential decisions, avoiding burden shifting and suboptimal reduction initiatives. Aalborg University is used as case study for this approach, and climate impact accounts from 2018 to 2022 have been developed. The study will investigate how decision makers at AAU understand and utilise the results from traditional guidelines and the new approach presented in this paper. The paper proposes potential improvements to more traditional accounting guidelines such as the Green House Gas Protocol and compares the climate impact accounts with traditional climate accounts based on the GHG-P. An example of an improvement proposed, is how to account for buildings, as traditionally the construction is only accounted for in the first year and maintenance and operations are accounted for in all remaining years. This traditional approach has two downsides, first it means that a building would be climate neutral if no maintenance or operations are conducted for a given year, secondly it disregards the impact of occupying said building. When an organization utilises its buildings they are removed from the market for buildings, which results in the construction of new buildings for other expanding organizations, whereas if an organization reduces their building mass, they release new building to the market, in turn displaces the construction of new buildings of the same type. This new proposed approach of impact accounting presents a new challenge, namely that the results and methods need to be clearly communicated to the relevant decision makers, so that they understand on which premises their decision are based. This issue is addressed through transparency both in how the emission intensities are calculated and a short but comprehensible description of how to interpret what these intensities represents.

#### **5.04.P-Tu496 ECOSCORE for Vehicles Procurement in Belgium: an Update in the Methodology to Integrate Life Cycle Assessment Approach**

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In Belgium, a tool called Ecoscore has been developed to help both for a more environmentally friendly procurement from consumers' side and for policy making. Ecoscore rates the environmental performance of vehicles based on a well-to-wheel approach. An Ecoscore between 0 and 100 is attributed to every vehicle. The closer to 100, the more environmentally friendly the vehicle is. All cars from the Belgian fleet have an Ecoscore. The current methodology was created in 2006 when most of the fleet were thermal passenger cars and only a few electric vehicles existed. Hence the choice for a well-to-wheel approach was sufficient to answer the goal of the score to rank the different cars of the Belgium fleet based on environmental performances. Currently, with the electrification of the fleet, the score methodology needs to be improved. This update is discussed with a steering committee composed of different stakeholders: university, research institute and the environmental agency per region of Belgium.

This study presents the challenges and work done to adapt the methodology of a tool that is used for consumers' procurement and policy making. Several alternative scores were assessed through different criteria such as ease of interpretation, completeness of impacts and differentiation of EVs (comparison between the score of a small city car and a large SUV). Some scores kept the same methods but added new life cycle stages inventories (i.e., battery production, glider production). Then,

other scores were considering all life cycle stages with a change in the methodology (i.e., ReCiPe 2016 or Environmental Footprint (EF) 3.1). The preferred method was EF 3.1 as it is the one preconized by the European Commission.

However, one main issue arises with the proposed methodology of using EF3.1. The main strength of Ecoscore is indeed that the database for exhaust emissions comes from homologation tests. It means that only for certain pollutants for the damage categories of EF3.1, the exact measures for every car in Belgium are available. For this reason, it has been decided to adapt the EF 3.1 method to only emissions included already in Ecoscore and their impacts on the corresponding damage category from EF 3.1.

Therefore, the new score is based on an adapted EF3.1 methodology. Further research will consist of validating the new method. The representativity and relevance of it will be assessed, especially regarding the effect of normalization and weighting choice.

#### **5.04.P-Tu497 Improving the robustness of freshwater ecotoxicity impact assessment of cosmetic products in life cycle assessment: summary and illustration of the work conducted by the EcoBeautyScore Consortium**

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Life Cycle Assessment (LCA) has been recognized by the European Commission (EC) as the most effective method to assess the overall environmental footprint of products and services. The EC initiated the Product Environmental Footprint (PEF) project in 2013 to harmonize application of LCA for the determination of environmental footprints of products on the European market. The PEF guidance is considered today as the reference measurement system in Europe regarding environmental footprint assessment of consumer products.

The EcoBeautyScore (EBS) Consortium, grouping more than 70 companies or professional associations of the cosmetic industry at worldwide scale, was launched in 2021 to develop a common environmental footprint measurement and scoring system for cosmetics, based on LCA and aligned with the PEF method, enabling communication to allow consumers to make more environmentally informed purchasing choices. However, Consortium members acknowledge that the PEF as a reference method is not fully workable for the Beauty sector, improvements being needed for cosmetics regarding methodology. One of the key topics subject to adjustments from PEF in the EBS method is the impact assessment method for freshwater ecotoxicity.

The PEF method prescribes the use of the USEtox<sup>®</sup> 2.1 model adapted by the Joint Research Center (EC) for freshwater ecotoxicity impact. There is a consensus within the EBS Consortium on some limitations of this adapted USEtox model for freshwater ecotoxicity and the corresponding characterization factors (CF) (as part of the EF 3.1 method package) needed to generate robust environmental scores of cosmetics suitable for the main objective of the Consortium, *i.e.* meaningful differentiation between products. The actions achieved within the EBS Consortium to tackle these limitations and improve the robustness of the freshwater ecotoxicity CF will be presented along with practical cases showing their relevance for application to cosmetics. A particular focus will be placed on: (1) the alternative method proposed to calculate the Effect Factor of CF based on the Most Sensitive Species approach, considered as more robust than the Species Sensitivity Distribution approach for cosmetic ingredients having a small number of ecotoxicity data on different aquatic species, (2) updated CF using more recent ecotoxicity data and (3) replacement of inconsistent CF of the EF method package with improved ones.

#### **5.04.P-Tu498 Will digital memory technologies decrease their environmental impacts? The memristor's environmental footprint**

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The use of digital technologies is a daily practice in human lives. According to one of the latest reports of the International Energy Agency, data centers and data transmission networks are responsible for 1% of energy-related GHG emissions. The Waste Electrical and Electronic Equipment European Directive regulates the end-of-life of the devices and obliges companies and public institutions to treat, reuse, recover and recycle electrical and electronic devices. The European Commission identified servers and data centers as environmentally concerning fields, and compiled standards that estimate their energy consumption.

The next generation of digital non-volatile memory devices, memristors, has a strong position to replace current technologies in the future due to their scaling potential, similar retention times, lower power consumption and faster latency. In this study, we show the results of applying Life Cycle Assessment (LCA, complying with standards ISO14040 and 14044) to three

different memristor technologies, i.e., halide perovskite memristors (Ps-M), TiO<sub>2</sub>-based memristors (Ti-M) and hafnium-oxide-based memristors (Hf-M).

The report shows their cumulative energy demand and ReCiPe methodology results for several impact categories per memristor area and functional unit (FU). FU consist of a combination of their figure of merits, i.e., area and energy required for a device (memristor) capable of storing 1 bit, considering a switching time of  $1.5 \times 10^{-4}$  seconds per storage cycle and per second. The main conclusions are: 1) Electricity and direct emissions during manufacturing are the primary factors driving life-cycle impacts. 2) Regarding Ps-M, the gold layer and the photolithography process have the most significant impact. 3)Ti-M has a more straightforward manufacturing process, and its most significant impact is observed in the semiconductor layer. 4) Hafnium-oxide memristors are the ones with the lowest environmental impact and promising features. 5) Ps-M and Ti-M memristors are produced at a laboratory scale and still exhibit low retention times to be competitive in the market. 6) More retention times and cycles would be required to compete with Hf-M ones.

#### **5.04.P-Tu499 Life Cycle Assessment applied to novel solar modules' encapsulants**

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The assembly of photovoltaic (PV) modules requires a significant amount of materials, with the cover glass contributing considerably to the environmental impacts and to the overall economic cost. The currently prevailing cover material for PV modules is 3-mm-thick glass, representing 10% to 25% of the total cost. It is essential to enhance the mechanical properties of glass for PV modules to achieve a thickness reduction below 3mm. This decrease will directly influence the total weight of PV modules, which typically range from 20-25kg for a standard module. Glass accounts for approximately 75% of the weight in a framed crystalline silicon module and exceeds 95% in a frameless thin film module. Reducing weight is crucial for facilitating the transportation, installation, and decommissioning of PV modules. Therefore, there is a need to reduce the thickness of the glass cover while simultaneously improving the service lifespans and efficiencies of PV modules.

The strength and toughness of flat glass are primarily influenced by surface properties rather than the bulk characteristics of the glass. Reducing thickness is accomplished through reinforcing and incorporating flexibility in the glass. There are three fundamental approaches to enhancing glass's toughness and fracture energy. These basic strategies include thermal or relaxational toughening, chemical (ion-exchange) toughening, and intrinsic toughening.

Adding of ions is crucial for glass's mechanical and optical enhancement for PV applications. Using iron oxides incorporated into the glass for UV filtering functions unavoidably results in absorption in the visible (Fe<sup>3+</sup>) and near-infrared bands (Fe<sup>2+</sup>). In contrast, outcomes with bismuth (Bi<sub>2</sub>O<sub>3</sub>) show promise, demonstrating effective UV filtering capabilities while maintaining the glass free from absorption in both UV and IR bands. The production cost is two to six times higher than conventional thermally toughened glass.

The report presents an application of the Life Cycle Assessment methodology to determine if strengthening Soda-Lime-Silica (SLS) glass through Na<sup>+</sup>/K<sup>+</sup> ion exchange, and to include Bi<sub>2</sub>O<sub>3</sub> for a more efficient UV filtering and durability is worthwhile from economic and environmental perspectives. Although this process is more expensive, it could be beneficial in the long run as the glass becomes more resistant, potentially lasting longer over time and exhibiting improved efficiencies by enhancing its optical properties.

#### **5.04.P-Tu500 Environmental Impact of Municipal Wastewater Management System Based on Life Cycle Assessment (LCA) in Indonesia**

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Water quality in an area has an important implication on human and environmental health. Urbanization and lack of proper municipal wastewater treatment coverage has left the water condition in Jakarta, Indonesia in a heavily polluted condition. Proper municipal wastewater treatment, so called Centralized Wastewater Treatment Plant (WWTP), has a low treatment capacity coverage in Jakarta. Based on the data from UNICEF in 2017, only around 7% of domestic wastewater in Indonesia that has been treated with proper method [1]. Septic Tank is the most often used municipal wastewater treatment, installed personally by each household. Wastewater leakage from the septic tank is a common issue due to the lack of maintenance. Water pollution is getting worse since open defecation or direct discharge activity is still happening although at the low percentage. Based on the information from the World Bank in 2022, still there are around 4% population in Indonesia that is still practicing open defecation [2]. Environmental impact assessment for each municipal wastewater treatment: Centralized WWTP, Septic Tank, and Open Defecation (direct discharge) should be carried out in Jakarta. The result of the environmental impact assessment can be used as the basis for policy making in terms of municipal wastewater management in Jakarta. In this study, the evaluation is conducted for three types of municipal wastewater treatment method: Centralized WWTP, Septic Tank, and Direct Discharge. The simulation was carried-out with SimaPro using the ReCiPe2016 model. Greenhouses Gasses (GHG)

was chosen as the impact category to be analyzed with since it gave the significance environmental impact in terms of municipal wastewater management.

#### **5.04.P-Tu501 Systematically Assessing Environmental Impacts of Pharmaceutical Products – Lessons Learned**

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During the life cycle stages of healthcare processes and products, many emissions and resource extractions occur that can have negative health and environmental effects. If one person is cured today, at the expense of making other people sick tomorrow, healthcare becomes counter-productive. Safe and sustainable pharmaceuticals can contribute to a healthy healthcare system. To understand the environmental consequences of pharmaceuticals, Life Cycle Assessments (LCAs) can provide valuable input. However, it is not clear to what extent these studies are complete (i.e. covering all life cycle stages and important environmental impacts), representative for global pharmaceutical production and consumption, and accurate for impact prediction, accounting or monitoring.

Therefore, the goal of this work is to review scientifically published LCAs of pharmaceutical products, to come to recommendations for complete, representative, reliable, and accurate application of LCA to pharmaceutical products. We do this by presenting the current state of the science of LCAs of pharmaceutical products whereby methodological choices, knowledge gaps and challenges are identified. We conclude with recommending solutions on how to deal with challenges, strengths and limitations in pharmaceutical LCAs.

LCAs of 143 pharmaceutical products with a known active pharmaceutical ingredient (API) and 6 with an unknown API have been retrieved during literature screening. The LCAs on pharmaceutical products showed varying scopes, approaches and data quality. This limits comparisons and applications in broader environmental sustainability assessments.

Common inconsistencies in performing LCAs for pharmaceutical products are missing life cycle stages, unspecified inventories, a limited life cycle impact assessment, due to missing impact categories or characterization factors, and missing interpretation steps. To allow for complete and representative LCAs, comprehensive functional unit and system boundaries are needed. Transparent life cycle inventories lead to greater reliability, while complete impact assessments and interpretation contribute to representativeness of the LCA study. This presentation will show how identified solutions can be put into practice to aid decision-making.

#### **5.04.P-Tu502 Environmental Impact Assessment of Paint Production and its Distribution**

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Current societal pressure to have sustainable resource use and products reaches every economic sector. In the chemical sector, paint industries are not an exception. In past years, these industries changed their production processes and primary inputs to have non-toxic, safe products with a special focus on reduction of volatile organic compounds as concerns on human health raised. Concerns have extended to environmental aspects, making industries invest on innovation aiming to produce paints with lower carbon footprint, less resource use and biocompatibility. Achieving these aims requires deep understanding of how materials and processes across the value chain generate impact. In previous assessments of paint environmental impact, aspects included were: production of primary materials, their transportation to production plant, and production of paint, including aggregation of raw materials and packaging process. This assessment provides an insight on the most environmentally impactful decisions, focused on primary materials, leaving transportation and packaging aside due to their lower impact in comparison. Nevertheless, this assessment lacks the inclusion of stages once the product leaves the production plant. Aspects to be considered are the impact arising from paint that is discarded before reaching to the consumer (around 20% of paint produced), and the impact produced by the paint which reaches the consumer but is not fully used and spoils in houses (around 10% of paint produced). This discarded paint contaminates water and affects ecosystems. During this study we propose to extend the LCA to the entire supply chain, with the aim of finding the impact of waste paint and therefore propose suitable strategies to avoid this spoilage. Currently, discussions about biocides are in place, given that they can extend the lifespan of paints and prevent spoilage, but there is concern about their possible effects on biodiversity and health. Therefore, one scenario is created to investigate how the inclusion of biocides contribute to avoiding waste paint and if a trade-off is created between lifespan extension and effects on biodiversity. Additionally, the inclusion of a circular business model is investigated due to their potential to reduce waste. The findings will provide information for decision makers in companies and policy, so that they can push for right strategies in this sector and contribute to the decrease of gas and water emissions due to paint use.

#### **5.04.P-Tu503 Life cycle analysis for Environmental impact assessment of cavitation assisted valorization of keratin waste**

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In recent years, Acoustic cavitation and hydrodynamic assisted processes are the prominent techniques used for process intensification in various industries such as textile, pharmaceuticals, polymer, food, agricultural and petroleum. In various



studies, researchers have reported the reduction of energy consumption due to cavitation assisted processes (CAP) compared to conventional processes (CP) indicating that CAP could be considered as a “sustainable” alternative. However, the environmental impacts of these processes have not been compared. Indeed, till now very few studies have reported results to indicate the effective reduction of environmental impacts of CAP, generally considered as green, in comparison to CP. Thus, it needs to be evaluated at early development stages through environmental assessment tool i.e. life cycle assessment (LCA). Performing LCA will provide a better understanding of factors with environmental impact associated with both CAP and CP at early development stages. The aim of this work is to elucidate the potential of cavitation for reducing environmental impact associated with the cavitation assisted valorization of keratin waste and its comparison with the conventional processes. For this purpose, a gate-to gate LCA analysis is carried for the processes without and with cavitation. Different categories of environmental impact considered in the current study includes greenhouse effect, eutrophication, acidification, ozone layer depletion, ecotoxicity and human health. The results of the current study will enable the quantification of potential environmental impacts of CAP and hence their sustainability before they can be industrially up-scaled.

#### **5.04.P-Tu504 Environmental impact of alternative red mud utilization for metal extraction**

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Red mud is the alkalic suspension waste of red to brown colour created during the primary aluminium production process. With the typical pH range about 10 to 13, red mud is considered hazardous waste and high amounts of acids are needed for the neutralization process. The suspension contains particles of size lower than 10 micrometres which resolves in extremely low sedimentation rate and long-time deposition in specially designed sedimentation reservoirs. The particle size poses also a significant problem for further processing. Per 1 ton of primary aluminium there is 1.6 to 3.7 tons of red mud produced. In 2020 worldwide production of red mud was about 175 megatons. Fe<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub> are representing most of the weight of red mud. CaO, TiO<sub>2</sub> and Na<sub>2</sub>O also usually accounts for significant weight percentage.

While several solutions to the red mud treatment were introduced in laboratory scale none of them has been adopted by the aluminium industry. The main reason being the unfavourable economics of invented processes. ABTOMAT project aims to extract Fe from red mud through the gasification process and to extract REEs, Sc, Ti, Fe, V and alkali metals from the after gasification red mud residue. The red mud samples from the ETI Aluminium plant in Seydişehir, Türkiye were used in this study as input materials. The XRD analysis of input red mud identified around 30% of the weight as Hematite (Fe<sub>2</sub>O<sub>3</sub>) and more less the same percentage as Quartz (SiO<sub>2</sub>), around 15% of weight as Calcite (CaCO<sub>3</sub>) and similar content of Alumina (Al<sub>2</sub>O<sub>3</sub>).

This paper is focused on the life-cycle assessment of the red mud utilization for extraction of valuable metals and environmental impacts of experiments with red mud utilization involved in ABTOMAT project. Environmental assessment and indicators are determined using the life cycle assessment method (LCA) with the implementation of the European methodology, PEF 3.1, i.e., Product Environmental Footprint. The PEF 3.1

The goal of the study is assessment of environmental impacts of current situation of red mud management and calculating the impacts with implementation of the utilization using gasification. By using modelling software and data calculations we identified crucial processes and flows. We established potential added value of the new utilization in the treatment of aluminium red mud and were able to compare it with present situation as base level.

#### **5.04.P-Tu505 Human toxicity emissions produced by copper supply chain: A comparison between different producer countries**

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Copper stands as a pivotal metal in modern industry due to its electrical and thermal properties, ranking third in global metal utilization. Key sectors relying on copper include construction and equipment manufacturing, accounting for more than half of worldwide copper consumption. Meeting the global copper demand primarily depends on deposits concentrated in Latin America, notably in Chile and Peru, totaling 271 million tons by 2023. As global copper production continues to rise, driven by population growth and the economic advancement of previously industrializing nations, the study's objectives are to assess countries deeply involved in copper activities and to comprehend the global copper flow. Additionally, the study aims to compare countries to identify the one with the most significant impact on human toxicity in copper production. The methodology employed for Life Cycle Assessment (LCA) relies on input-output matrices, an economic tool measuring the impact of changes in demand for goods and services. This matrix serves not only as an economic tool but also finds application in life cycle analysis. The chosen methodology, "Environmentally-Extended Multi-Regional Input-Output Analysis (EEMRIO)," allows for a comprehensive study of the origin of impacts. According to United States Geological Survey (USGS) data, Chile leads in copper mining production, while China dominates copper metallurgy. Analyzing human toxicity emissions from major producers in each region (Chile in South America, the United States in North America, Poland and Spain in Europe, Congo in Africa, China in Asia, and Australia in Oceania), the results indicate that China has the highest impacts on

human toxicity in both mining and metallurgy. Spain, in contrast, boasts the lowest emissions in mining, while for metallurgy, Congo has the lowest cancer emissions, and Poland has the lowest non-cancer emissions.

#### **5.04.PC LCA in Policy, Decision-Making and Communication to Support the Transition Towards Sustainable Consumption**

##### **5.05.A Life Cycle Impact Assessment – Advances in Modelling and Application**

###### **5.05.A.T-01 Biodiversity Impact Assessment for the Financial Sector: Guiding Investments and Policies**

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Biodiversity loss, driven by human activities, significantly affects the environment, human societies, and economies. This study examines the role of financial institutions in addressing these challenges. Using the Environmentally-Extended Multi-Regional Input-Output Assessment (EEMRIO) and LCA techniques, we offer insights into how these methodologies can be used to inform financial decisions related to biodiversity focusing on three key aspects: biodiversity impacts, ecosystem service dependency, and net impact. Our method combines spatially explicit characterization factors from LC-impact with the GLORIA database to estimate biodiversity impacts. As a case study we assess the biodiversity impact of the MSCI ACWI stock index which consist of about 3000 large and mid-sized companies, from 23 Developed and 24 Emerging countries. The results demonstrate most of the biodiversity impact is caused in the Americas, followed by Asia, despite its low representation in the index's country weight (6%). Europe shows the least impact. These results emphasize the need to account for global supply chain linkages as products sold in one country might have significant biodiversity impacts elsewhere due to sourcing of production inputs. Secondly our results identify the main determinants of the index's impact: land use, followed by water stress and climate change. Although a majority of the impact is localized in few sectors, the distinct characteristics of these sectors require industry-specific mitigation approaches. We also analyse dependencies on ecosystem services by coupling ENCORE data with GLORIA in order to analyse double materiality. The combined analysis shows both, the influence companies have on biodiversity and the reciprocal effects. Companies neglecting these impacts risk financial setbacks, making it a crucial concern for investors and policy makers.

###### **5.05.A.T-02 Linking freshwater ecotoxicity to species loss in life cycle impact assessment**

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Linking predicted ecotoxicity impacts to damage on ecosystem quality as an area of protection in life cycle assessment (LCA) involves using a severity factor that converts 'affected' into 'disappeared' fractions of exposed species. A relationship of 1:1 has been reported between predicted effects (msPAF: multi-substance Potentially Affected Fraction of species) and observed impacts (PDF: Potentially Disappeared Fraction of species) calculated from acute EC50 effect concentrations. However, the recent adoption of a 20% effect threshold for use in LCA ecotoxicity characterization requires a PAF-to-PDF linkage at the HC20<sub>EC10</sub> level (HC20: hazard concentration at which 20% of species are affected above their chronic EC10 effect concentration). To address this gap, we introduce a systematic approach that consists of a stepwise procedure to calculate mixture toxicity pressure (msPAF) at the level of chronic EC10 equivalents (msPAF-EC10eq), to evaluate non-collinearity with other abiotic factors and to characterize the observed "lost fraction" of species at 20% msPAF change. Our approach is used to derive data-driven severity factors for LCA at HC20<sub>EC10</sub> as a 'working point'. We started from an extensive Netherlands freshwater (bio-)monitoring data set and first calculated msPAF-EC10eq of ambient mixtures for all sites. Second, we established low variance inflation factors between toxic pressure and six other abiotic factors, suggesting that we can derive the PAF-to-PDF link with no or limited bias. Third, we visualized raw invertebrates' data patterns for biodiversity metrics. Fourth, we established PAF-to-PDF relationships through stepwise evaluation, binning msPAF data into 10 groups, excluding opportunistic species, calculating Observed Disappeared Fraction (ODF) of species and delta msPAF between groups, taking group 1 (i.e., 'pristine' water) as reference, and finally using a generalized additive model to predict the PDF at a 20% change in msPAF. Results show varied mixture toxic pressure (0 to 0.75) across sites, independent of other abiotic factors. The GAM model shows that a 20% change in msPAF-EC10 covaries, with 19% of initially-present species disappearing. Thus, we determined a consistent extrapolation factor of a near 1:1 relationship between predicted effects and actual damage required for different decision-support tools, such as LCA.

Keywords: mixture toxic pressure; species loss; life cycle impact assessment

###### **5.05.A.T-03 Towards a Unified Spatial Framework to Quantify Biodiversity Effects in Life Cycle Impact Assessment**

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With the increasing concern about global biodiversity loss due to increasing pressures on the environment, it is crucial to accurately characterize biodiversity effects in Life Cycle Impact Assessment (LCIA). Currently available multi-pressure LCIA methods, including the widely used ReCiPe and LC-IMPACT methodologies, quantify biodiversity loss per pressure-impact

pathway independently and at various spatial scales, challenging consistent aggregation of pressure-specific impacts to total biodiversity impacts. Although LC-IMPACT harmonizes pressure-specific biodiversity loss at various (local to regional) spatial scales to a global potentially disappeared fraction of species, no method quantifies combined effects of pressures harmonized to local, regional, and global biodiversity loss.

We propose a consistent framework that quantifies combined effects of various pressures on terrestrial biodiversity loss on the local, regional, and global scale, to be implemented in the ReCiPe methodology. As a first step in the framework, the local biodiversity loss is quantified as the product of the local potentially disappeared fraction of species (LPDF) of the different pressures per grid cell. Subsequently, the LPDF can be scaled to the level of the ecoregion using a multi-pressure species-area relationship. As a third step, the regional PDF (RPDF) values of the different ecoregions are used to calculate the global PDF (GPDF) using endemism richness. At each scale, the biodiversity loss can be disaggregated based on the relative contribution of each pressure to the combined biodiversity loss. Finally, pressure- and scale-specific effect factors are calculated based on the pressure-disaggregated biodiversity loss per scale and the pressure magnitude.

In the presentation, we will show the application of the proposed modelling framework in which the combined effect of both land use and global warming on terrestrial biodiversity are quantified on three spatial levels. Characterization factors will be presented in terms of local, regional and global PDF per area of land use and kg of greenhouse gas emission. Finally, the presented study will highlight the differences in characterization factors between the new proposed method and the method currently used in ReCiPe and other LCIA methods.

#### **5.05.A.T-04 Operational Accounting of two Major Drivers of Marine Biodiversity Loss in Life Cycle Assessment (LCA) of Seafood Products**

*Aurore Wermeille, Gregoire Gaillet and Anne-Claire Asselin, Sayari, France*

The latest global assessment report of the Intergovernmental Panel for Biodiversity and Ecosystem Services (IPBES) identifies 5 main drivers of biodiversity loss for terrestrial, freshwater, and marine ecosystems. For marine ecosystems, the driver “Direct exploitation of biotic resources” is the largest, followed by “Land- and sea-use change”, “Climate change”, “Pollutions”, and “Invasive alien species”. Up-to-date methods of resource depletion (Hélias et al., 2023) and seabed impact assessment (Woods and Verones, 2019) have not been applied to case studies other than their respective proofs of concept.

We included those two pressures in an operational method, accounting for Resource Depletion and Seabed Impact on ecosystems. We applied it to a business case study, and we identified key parameters and data driving results. Our goal was to determine if it was feasible to include these key drivers of marine biodiversity loss – Direct exploitation and Sea-use change – in the biodiversity impact assessment of seafood products over their value chain and for the three biota (terrestrial, freshwater and marine). A prescriptive framework was also included to collect data and determine their quality.

The method encompasses four drivers of biodiversity loss calculated with LCA. Climate change and pollution were assessed with LC-Impact while direct exploitation and sea-use change were calculated using respectively Hélias et al. (2023) (resource depletion indicator) and Woods and Verones (2019) (seabed impact indicator). Field data provided by companies enabled us to apply it to a practical case study on frozen saithe fished in the North Sea, at landing. Seabed impacts were spatialized based on logbook.

The application of the resource depletion indicator highlighted the great role of bycatch species which, when vulnerable to fishing, may account for the majority of impacts despite low catches. Regarding seabed impact, case study highlighted two main parameters influencing impacts of a fishing métier: i) the trawled area (in m<sup>2</sup>) and ii) the vulnerability of marine ecoregion. Case study highlighted different orders of magnitude between pressures. Seabed impact indicator is dominant in our case study and more generally should be dominant for fisheries targeting sustainably managed resources with active bottom fishing gears. In the perspective of aggregation of pressures, homogeneity between PDF units must be achieved, through further research.

#### **5.05.B Life Cycle Impact Assessment – Advances in Modelling and Application**

##### **5.05.B.T-01 Regionalized Characterization Factors for Microplastics Emissions in the Marine Environment**

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Keywords: microplastics, marine environment, life cycle impact assessment, characterization factors, regionalization  
Microplastics (1 µm–5mm) concentrations in the marine environment are increasing throughout the years. Consequently, their potential impacts on marine biota are becoming more important. These include, internal and external wounds, false satiation, and even mortality. Therefore, it is important to assess these impacts in the holistic approach of life cycle assessment (LCA).

Previous initiatives have developed characterization factors (CFs) to be implemented in the life cycle impact assessment (LCIA). These initiatives lacked the consideration of various marine sub-compartments, and different fate mechanisms responsible for the distribution of MPs within the sub-compartments. In order to refine fate modelling necessary for the

development of CFs, most influencing parameters on the fate of MPs were identified as an initial step. Along with the physical properties of the particles (size, density, shape), environmental and oceanographic variables were proven to be influencing. Therefore, regionalization is important to account for the spatial variability of these factors, which is the aim of the present study. For 8 different polymers of three different shapes and five size classes, a rate **K** matrix [ $\text{day}^{-1}$ ] is developed. Within the matrices, compartment-specific sedimentation rates, for 20 riverine emission points along the global coastline, are quantified using a 3D tracking model (TrackMPD). Other mechanisms are quantified based on equations and models from the literature. Fate factors (FFs) [ $\text{kg per } k_{\text{emitted}}/\text{d}$ ] are then obtained from the **K** matrices. CFs are then obtained by multiplying the FFs with water and sediment exposure-effect factors for micro/nanoplastics potential physical impacts on marine environment. They are expressed in  $\text{PAF}\cdot\text{m}^2\cdot\text{year}/\text{kg}_{\text{in compartment}}$  for the LCIA impact category “physical impacts on biota”. Using MP input data from polluting rivers into the ocean, site-specific CFs are aggregated to obtain continental CFs. Consequently, continental CFs are aggregated into a global CF to represent the global impacts of MP emissions in the marine environment. These regionalized CFs are an improvement to already existing factors. They take into consideration the physical properties of the particles along with the spatial variability of environmental factors within various marine sub-compartments.

#### **5.05.B.T-02 Spatial assessment of the environmental sustainability impacts of pesticide use across Europe**

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To get a broader understanding of pesticide impacts on human health and the environment at the regional or country level, data representing pesticide use beyond specific farms are required. A systematic comparison of the environmental impact performance of different pest control options based on actual country-level data and factors to scale between farm-level and country-level data, putting a special emphasis on the direct field-application impacts of pesticides, is currently missing. To address this aspect, we adopted a spatially explicit environmental fate model and integrated it with widely applied life cycle toxicity characterization models. To characterize national-level impacts of pesticide use, actual pesticide use data are combined with physico-chemical substance databases to assess emissions of applied pesticides to crops at different crop growth stages and via different application methods to different environmental media. Resulting emissions are coupled with results from spatial multimedia modeling for environmental fate, using additional physico-chemical substance data, and spatial data for atmospheric, aquatic and terrestrial environments. The results are coupled with information on exposure and effects for humans and ecosystems as implemented in the global scientific consensus model USEtox. Concentration maps and related impacts were determined for Europe. On average, 5 tonnes of pesticides per year were applied per country, with a cumulative mass of 53 tonnes. For glyphosate, we reached high-end steady-state concentrations of  $25 \text{ mg}/\text{m}^3$  in freshwater, while overall highest concentrations were found in agricultural soil, reaching  $\sim 100 \text{ mg}/\text{m}^3$  for several pesticides. Human toxicity impact of considered pesticide use sums up to  $\sim 2$  million disability-adjusted life years, with France, Italy and Spain as main contributors, and with terbuthylazine, prothioconazole, prosulfocarb, metconazole and metiram contributing jointly to  $>83\%$  of total human toxicity impacts. Ecotoxicity impact sums up to 80 million species-years lost over the considered land area, again with France, Spain and Italy dominating. Across countries, spirioxamine, pendimethalin and azoxystrobin contribute jointly to  $\sim 75\%$  of ecotoxicity impacts, with highest impact in agricultural soil ecosystems. Our findings on the substantial damage from pesticide use show that urgent action is required to replace chemical pesticides with more sustainable alternatives across EU-countries.

#### **5.05.B.T-03 Highly Resolved Life Cycle Impact Assessment of Global Industrial Air Pollution**

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Health impacts due to particulate matter (PM) air pollution present a major threats to human well-being, but are difficult to capture accurately with life cycle assessment due to their highly regionalized characteristics. Drivers of PM air pollution are a number of criteria air pollutants such a primary fine particulate matter (PM<sub>2.5</sub>), sulfur dioxide (SO<sub>2</sub>), nitrogen oxides (NO<sub>x</sub>) and ammonia (NH<sub>3</sub>), which are dominantly being emitted from energy-intensive industrial sectors. This study consistently links state-of-the-art regionalized life cycle impact assessment (LCIA) with a broad range of emission measurements for these substances with site-specific technical data, emission models, and atmospheric fate and effect models to quantify health impact reduction potentials at nearly all global fossil power plants, steel mills, oil refineries and cement plants. Thus, emission hotspots and reasons for regional human health can be identified, which allows for targeted policy interventions. The results highlight the key role of emission abatement at coal-consuming industrial sites, especially in densely populated areas of Asia (Northeastern India, Java in Indonesia, Eastern China) and Western Europe (Germany, Belgium, Netherlands) as well as in the US. One of the global top priorities is the high amount of SO<sub>2</sub> emissions in India, which are caused by lack of flue gas treatment and contribute a particularly high share of local health impacts despite a limited number of emission sites. At the same time, the massive infrastructure and export capacity build-up in China in the recent years is a cause for major regional and global health impacts related to cement-making and steel manufacturing and thus requires more stringent regulation than in the rest of the world due to unfavorable environmental conditions and high population densities. The level of detail in this study shows how highly resolved LCIA methods and emission inventories can be combined to provide very specific reduction suggestions for environmental impacts. Furthermore, such work can also enhance standard LCA and carbon footprinting by informing the development of background LCI databases and by adding further depth to the analysis of existing studies.

#### **5.05.B.T-04 Incorporating Ecosystem Services in LCIA - Recommendations from GLAM 3**

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Current Life Cycle Assessment (LCA) practice has struggled to include Ecosystem Services (ES) in its life cycle inventory or into impact assessment.

As part of the third Global Life Cycle Impact Assessment Methods (GLAM) project, a task force was established to propose impact methods and endpoints for potential impacts ecosystem services and over the last 3 years the project has developed recommendations and build on existing methods to propose a common endpoint and recommended characterization factors.

After an extensive review six midpoint categories and four endpoint categories have been recommended. The midpoint categories included are Soil Organic Carbon(SOC) deficit, erosion resistance, groundwater regeneration, mechanical filtration, physiochemical filtration and soil salinization.

Endpoints were calculated for 4 of these pathways SOC deficit, erosion resistance, groundwater regeneration, mechanical filtration using economic valuation of ecosystem service loss compared to potential natural vegetation.

The method was normalized against annual global land occupation and transformation and established a total value of ecosystem service damage of \$1.7 trillion international dollars which compares to total value of agriculture in the world of approximately 4.4 trillion US dollars. Land occupation accounts for most of this impact with SOC deficit (0.97 Trillion) and ground water regeneration (0.58 Trillion) being the main contributing ecosystem services.

The paper has demonstrated a pathway for including an important group of ecosystem service impacts associated with land occupation and transformation. By finding a common endpoint for 4 of the indicators a practical summary indicator for including in ES impacts in the LCIA framework which is suitable for LCA based on existing data.

#### **5.05.P Life Cycle Impact Assessment – Advances in Modelling and Application**

##### **5.05.P-Mo469 Opening the Pandora's box of Soil Biodiversity in LCA: a First Attempt to Model the Impact of Human Activities on Earthworms**

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Current Life Cycle Impact Assessment (LCIA) methods are still lacking the assessment of the impacts of human activities on soil biodiversity. Recent estimates see soil organisms as the major component of global biodiversity accounting for nearly 60% of the planet's life (Anthony et al, 2023). For this reason, while evaluating the impacts of human activities on biodiversity, soil ecosystems should not be overlooked anymore. In this context, earthworms may represent a first good proxy for soil biodiversity, especially in relation to human activity impacts, as their sensitiveness to anthropic stressors is well known (Conrado et al., 2023).

The objective of this work is to present a first attempt at filling the identified knowledge gap by developing a life cycle impact assessment model to quantify the impact of land occupation and transformation on earthworms' diversity, compatible with the nomenclature system of the Environmental Footprint (EF) and aligned with the framework to assess the impacts of land use activities in LCIA established by Koellner et al. (2013). This work will build on progress made over the last years to assess the distribution of soil organisms at global scale.

The model developed will be fully regionalized, and in addition aggregated characterization factors (CFs) will be provided at country level and global level. The calculation of the CFs will be based on a number of global datasets, including maps of earthworm diversity (Phillips et al., 2019), land cover maps, and maps of climate regions and soil types. The developed CFs will be fully operational and compatible with life cycle inventories developed with the EF nomenclature. Examples of their application will be provided.

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##### **5.05.P-Mo470 Using Species Sensitivity Distribution to capture ecosystem scale fisheries impacts on biodiversity**

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Fishing is one of the main drivers of global marine biodiversity loss. The impact of fishing on ecosystem quality is a newly established impact pathway characterising impacts on individual stocks, but overlooking broader ecosystem-scale damages.

This work enhances impact characterisation by integrating indirect impacts on target and non-target species in the ecosystem. The new approach draws inspiration from the USEtox method of constructing Effect Factors for ecotoxicology impacts, using Species-Sensitivity Distributions (SSD) to fisheries pressure to quantify the ecosystem-scale impacts in a single Characterisation Factor (CF) value per target catch.

FAO regionalised catch data and the dynamic ecosystem model Ecopath with Ecosim model the cause-effect relationship between fishing pressure, trophic interactions and the proportional abundance of each fish stock present. Akin to the effect-concentration curves that determine toxic effects of contaminants, biomass depletion curves reflecting the species-specific responses to increasing fishing pressure are compiled. SSDs per target species are constructed based on a threshold depletion value, to generate ecosystem-scale impact values in Potentially Affected Fraction of species (PAF) units.

The depletion curves are computed relative to a reference state: the carrying capacity of the stock- representing a hypothetical pristine state from which biodiversity loss can be measured. CFs are ultimately derived from the PAF curves per target species, following the USEtox approach but using current catch pressure to establish a “HC50 equivalent” point.

In contrast to contaminant effects, depleting a target species can positively affect other species in the ecosystem by altering predation pressure. Defining a dual threshold analogous to the (single) EC20 in ecotoxicology allows both negative and positive population changes relative to the reference state to be considered within the potentially affected fraction of the population.

To validate this new approach, 41 novel midpoint CFs are computed for Functional Groups in the Adriatic Sea.

The methodology offers significant improvements by accounting for indirect impacts via altered ecosystem dynamics, capturing both over-exploitation and over-expansion of populations resulting from fishing, and using Functional Groups to broaden representation of ecosystem effects, demonstrating a more comprehensive assessment of fishing impacts on biodiversity.

#### **5.05.P-Mo471 Towards a consistent assessment of potential impacts of human activities on the instrumental value of natural systems in life cycle assessment**

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The concept of "ecosystem services" (ES) encompasses the “benefits populations derive, directly or indirectly, from ecosystem functions”. Recent ES classifications includes both services offered by living systems and geodiversity.

Integrating ES into policy and decision-making processes is imperative to prevent their erosion. Integration of ES into Life cycle assessment can be done within the impact assessment phase. However, there is no harmonised framework for assessing potential impacts on resources and ES, leading to inconsistent development of characterisation models, partial coverage and/or double counting. This research addresses this challenge and provides a conceptual framework for characterizing the potential impacts of human activities in terms of changes in natural systems’ instrumental value. It builds on the research of the ES community and provides a consistent framework that intertwines resources within ES and clarifies the terminology. The position of impacts on ES in relation to other more common AoP (e.g. Human health and ecosystem quality) is discussed.

A conceptual framework is presented to depict potential impact mechanisms on natural systems' instrumental value. The framework is derived from existing LCIA frameworks and incorporates the cascading nature of ecosystems. Human interventions can induce modifications at various levels of the cascade model. Adaptation and non-adaptation responses lead to two new Areas of Protection (AoP). The *Ecosystems and resources' services (ERS)* AoP accounts for the potential loss of nature’s instrumental value if deployed adaptation measures are inadequate to balance the loss of service. The *Human assets* AoP accounts for the monetary valuable assets used to avoid a loss of service (adaptation or replacement costs), or due to the loss of valuable socio-economic assets (e.g damage on infrastructures). Insufficient adaptation can induce potential impacts on human health and ecosystem quality. A monetary unit is proposed to quantify damage, adaptation, and replacement costs. However, these scores cannot be summed due discrepancies between monetary valuation methods.

The framework reconciles impact modelling for resources and ES, clarifying terminology, promoting consistency, and guiding future LCIA developments. It emphasizes the complexity of ES and their interconnectedness with other AoP, providing a comprehensive approach for assessing human activities' potential impacts on nature’s instrumental value.

### 5.05.P-Mo472 Weighting Factors for LCA – A New Set from a Global Survey

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The Weighting subtask of the United Nations Environment Programme (UNEP) Life Cycle Initiative's "Global Guidance for Life Cycle Impact Assessment Indicators and Methods" (GLAM) project work will be presented. This work provides global weights (weighting factors) for the three endpoint impact categories (Areas of Protection, AoPs) human health (HH), ecosystem quality (EQ), and natural resources and ecosystem services.

**Methods** A Discrete Choice Experiment (DCE) elicited the preferences of respondents through interviews and a web survey. Responses were obtained from a subset of countries pertaining to each income level defined by the World Bank (i.e., low, lower-middle, upper-middle, and high) and used to calculate adimensional (between 0 and 1) weights using two different approaches: econometric and Multiple Criteria Decision Analysis (MCDA). The econometric approach is grounded in random utility theory and its weights are obtained by transforming the estimated preference parameters from a multinomial logit model. The MCDA approach is grounded in deterministic value theory and its weights represent the vectors that best reconstitute the choices of each individual, using linear programming to fit an additive value function.

**Results** The weights calculated are presented and discussed, including income group population share adjustment of the weights. Recommendations for the use of these weights are also provided, as well as comparison with other existing weights. The econometric and MCDA disaggregation approaches give similar weights. For high income countries EQ has the highest weight; for upper-middle-income countries EQ and HH have the same weights using the econometric approach, while in the MCDA approach HH is weighted higher than ecosystem quality. For the two lower income country groups the priority is given to HH with both approaches. The sets of weights presented in this paper are also related to existing LCA and environmental science and economics frameworks.

**Conclusion** Weighting is an optional, subjective part of LCA methodology. The weights proposed in this paper can coexist with other weighting proposals; they do not represent an objective truth, but reflect the results obtained from the large scale survey described in this paper. The weights are offered as an option for problem-owners or researchers who wish to use weighting to synthesize their LCA results, but do not have the resources to calculate them through other means.

### 5.05.P-Mo473 Recommended model for the endpoint assessment of mineral resource use in GLAM3 project

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The GLAM project led by the UNEP Life Cycle Initiative has been dedicated to developing guidance for Life Cycle Impact Assessment models based on international consensus. During the 3rd phase of the project, experts gathered to develop recommendations on LCIA indicators for all AoPs. A taskforce on natural resources (TF) aimed to describe generic impact pathways for natural resource use and recommend an impact assessment model, based again on review of existing models.

The generic framework of impact pathways from the previous study was revised, and a set of 23 models shortlisted for feasible fit with the generic framework. Two of the methods selected for evaluation are LCI methods, which enable classification (JRC-LCI, ARP) and inclusion (JRC-LCI) of some dissipative flows. The shortlisted methods were evaluated using a four-way rating against the following seven criteria, each with their own acceptance threshold based on priority and significance in the scope of GLAM3 project.

For mineral resources use, we updated FWL CFs using latest available market price data together with a measure of market price uncertainty. The FWL model uses the difference between social and market discount rates applied to market prices as a measure of damage to mineral resources. Market price is a most influential parameter of the CFs. While CFs for different

natural resources range from 32,960 USD/kg to 0.00180 USD/kg, by comparison the range of uncertainty of the CFs is small. The timeframe of assessment is, however, a most influential parameter of the CFs if it is set to less than 300 years. As for the normalization values derived from multiplying the CFs with the annual production of minerals, the CFs of fossil fuels are smaller than those of metals, but fossil fuels account for 72% of the normalized total due to faster extraction.

Application of the FWL CFs to inventory flows of mineral resources extracted from ground is currently the most practical approach due to the availability of these flows in standard LCI datasets. However, the values of minerals will not be lost when they are extracted but will be lost when they become inaccessible. The TF is also investigating application of FWL CFs to the dissipative mineral resource flows indicated in the generic framework of impact pathways through new classification approaches and adaptation of life cycle inventories.

#### **5.05.P-Mo474 Refining the Modelling Approach for Terrestrial Acidification in Life Cycle Impact Assessment**

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Terrestrial acidification, driven predominantly by anthropogenic activities, poses a threat to ecosystems by altering soil pH and impacting biodiversity. This study addresses the critical need for an updated modelling framework to generate spatially differentiated characterization factors (CFs) for terrestrial acidification.

The approach, based on the fate-effect framework from Rosenbaum et al. (2007), and more specifically on the work from Roy et al. (2014), quantifies global species richness decline of vascular plants, expressed as Potential Disappeared Fraction species (PDF) per kilogram of acidifying substance emitted.

Fate factors ( $FF_{i,j,p}$ ) account for dispersion, chemical reactions, and transportation of pollutants ( $NO_x$ ,  $NH_3$ ,  $SO_2$ ) from emission to reception, generating source-receptor matrices (Roy et al., 2012b). Sensitivity factors ( $SF_{j,p}$ ) estimate soil pH changes due to a marginal increase in the deposition rate of acidifying substances (Roy et al., 2012a), while effect factors ( $EF_j$ ) depict regional vascular plant species loss following a pH decline (Gade et al., 2021). Regional CFs, adjusted with Global Extinction Probability weighting, assess biodiversity loss globally (Verones et al., 2022).

Results provide regionalized CFs for 867 ecoregions and 206 countries, expressing the global loss in species richness due to marginal emissions of acidifying entities. Fate factors reflect expected patterns with highest values near emission sources (Roy et al., 2012b). Effect factors vary significantly at ecoregion level, influenced by vegetation types (Gade et al., 2021). As for sensitivity, soil pH responses to additional deposition can be linked to soil composition and local climate conditions.

This comprehensive modeling framework enhances assessments of regional and global impacts, marking an important step towards consistent evaluations of ecosystem quality.

#### **5.05.P-Mo475 Biodiversity Loss by Water Consumption in Global Watersheds: Effects on Riverine Fish Species**

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Human activities in the Anthropocene have caused a four-fold increase in global water demand, severely impacting freshwater ecosystems, and leading to an 84% decline in the global population of freshwater species. Despite covering only 0.01% of the Earth's surface, freshwater ecosystems host 9.5% of globally recognized animal species. In water ecosystems, persistent threats include flow modifications and alterations in river discharge. About 65% of global river discharge faces moderate to high threats, with a limited focus on biodiversity conservation due to data limitations. Within the context of life cycle assessment (LCA), the evaluation of biodiversity loss and ecosystem quality is a continuous effort. For this purpose, numerous methodologies have been dedicated to addressing factors like land use shifts, climate variations, environmental contamination, and water utilization. Another aspect involves evaluating environmental impacts on freshwater ecosystems due to water consumption, utilizing methods such as habitat simulation for LCA. In this study, the aim is to assess the impact of water consumption on global riverine fish species using characterization factors developed for this purpose in combination with detailed, spatially explicit inventory time series, from 2000 to 2016. The watershed level water consumption data is obtained from the WaterGAP 2.2d hydrological model. The impacts are calculated using both, the weighted average and maximum values of water consumption, to discuss the uncertainty in the results. This investigation seeks to contribute insights into the impacts of human freshwater use on riverine fish species and identify realted hotspots of species loss.

#### **5.05.P-Mo476 Enhancing Local Biodiversity Assessment: Similarities and Differences Among Methods Applied to Agribalyse Datasets**

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Primary drivers of biodiversity collapse, as identified by the Intergovernmental Platform on Biodiversity and Ecosystem Services, include land-use change, climate change, pollution, natural resource use, and invasive species. This work specifically addresses the "land use change" pressure on biodiversity associated with agriculture. The work addresses one of the REVALIM roadmap priorities. It assesses agriculture's impact on local biodiversity using case studies from the French Agribalyse database. Four methods (HCF (Kuipers et al. 2021), LUIS (Chaudhary and Brooks 2018), BVI (Lindner et al. 2019), BSS BioSyScan) were chosen from a qualitative assessment based on environmental relevance, completeness, transparency, reproducibility, acceptability, and feasibility. They were more thoroughly reviewed, and twenty crop and animal production datasets from conventional or organic agriculture were selected to test them.

The methods exhibit differences in approach, metrics, and specificity. HCF, LUIS and BVI methods are based on a classic LCA framework, and study the (potential) biodiversity variations linked to the product. HCF and LUIS methods rely on species richness, BVI uses the concept of hemerobia on a unit area for a time unit, while BSS determines unitless biodiversity points. Divergence occurs in biodiversity evaluation scales. HCF aligns with GLAM 1, proposing regional or global assessments, while LUIS proposes only a global level. HCF method introduces the Global Extinction Probability, combining species richness, vulnerability, and endemism. Consideration of habitat fragmentation, transformation and land management practices varies among methods. Geographical location proves significant in some methods, with considerable differences depending on the sub-criteria taken into account endemism, species vulnerability, roadless area, ... HCF and LUIS methods are easily applicable to Agribalyse, requiring minimal information, while BVI and BSS pose challenges due to limited agricultural practice data.

The study acknowledges biodiversity's multidimensional nature, highlighting the complexity of capturing it with a single indicator based on current methodological developments. It underlines the need for multiple biodiversity indicators in LCA to comprehensively represent product and supply chain impacts on biodiversity. It also highlights the current lack of inventory data, especially at landscape level (eg. SNH field size, ...), needed for BVI and BSS methods.

#### **5.05.P-Mo477 New Effect Factors for Freshwater Ecotoxicity of Pharmaceuticals**

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Pharmaceuticals play a crucial role in protecting and improving human health, but their release into the aquatic environment has raised significant concerns. Unfortunately, limited information is available regarding the risks of pharmaceuticals for the environment, mainly due to lacking data on the ecotoxicity of pharmaceuticals.

Life cycle assessment (LCA) is a widely used method to quantify the environmental impacts of products or and services. In LCA, Life Cycle Impact Assessment (LCIA) is the third step that quantifies and assesses the magnitude and importance of the potential environmental impacts of a product system. For this, Characterization Factors (CFs) are used, transforming the life cycle inventory data into environmental impact scores on various impact categories such as freshwater ecotoxicity. The calculation of CFs involves the effect factor (EF), translating ecosystem exposure concentrations into a measure for the potentially affected fraction (PAF) of exposed species. The derivation of EFs depends on the availability of ecotoxicity data, which is limited for pharmaceuticals. As a consequence, CFs have only been derived for less than 150 pharmaceuticals. This study aims to refine the approach to derive EFs for freshwater ecotoxicity of pharmaceuticals by optimizing the use of available ecotoxicity data. The method is illustrated by deriving new EFs for 217 pharmaceuticals, thereby significantly expanding the application domain of LCA for pharmaceuticals.

Our approach uses hazardous concentration HC20 from the species sensitivity distribution curve of chronic No Observed Effect Concentration (NOEC). Three main steps are followed to derive EFs: collecting all NOEC data, constructing the SSD with chronic NOEC and (iii) calculating the EFs. The database includes 217 pharmaceuticals from three sources.

Six of the top ten pharmaceuticals with the highest EFs, indicating the lowest HC20 values, are hormone-based. Among 11 pharmaceuticals with literature EFs, 9 have higher EFs in our approach, consistent with the expectation that chronic NOEC values are lower than EC50 values.

The present study proposes a refined approach to derive effect factors EFs for freshwater ecotoxicity, tailored to pharmaceuticals. New EFs were calculated for 217 pharmaceuticals. This will enable a more comprehensive evaluation of pharmaceuticals in LCA.

#### **5.05.P-Mo478 Are We Overestimating Marine Eutrophication Impacts in LCA? – A Temporal Approach to Assessing Nitrogen Emissions**

*Montserrat Nunez, IRTA, Spain*

No research has focused so far in LCA on studying the temporal dynamics of the processes that mediate in marine eutrophication. Temporality is important to predict intra-annual peaks of potential impacts due to use of nitrogen (N) fertilisers

and other N sources and introduce corrective measures in the timing of N emissions accordingly. Here, I show how I included temporality in state-of-the-art characterisation factors (CFs) for marine eutrophication impacts, which I derived at watershed level for the world and per quarterly periods in a year. CFs are for dissolved inorganic nitrogen (DIN) emissions. DIN is the largest fraction of N in rivers, particularly in human dominated watersheds and is also the most readily fraction of N assimilated. The CFs build upon fate factors of the state-of-the-art marine eutrophication model (Cosme et al.). They express the amount of time the fraction of the original DIN emission will be in the coasts each quarter of the year, which is the period within which DIN can be transformed in new algae and lead to biodiversity loss. I modelled three emission pathways: DIN to soil (to characterise emissions from fertiliser application), DIN to freshwater (to characterise any inland emission different than fertiliser application), and DIN to the coast (to characterise emissions to coastal waters). My CFs model the same fate, transport, and retention processes as the current state-of-the-art model plus DIN retention in lakes. Furthermore, I greatly improved the robustness of coastal water residence times and DIN removal in coasts. I achieved this by replacing old data from scattered studies and expert judgment used in Cosme et al. by recent and coherent data drawn from a coupled global ocean physical-biogeochemical simulation model at monthly time scale. Results show that annual quarters with higher rainfall and cooler temperatures lead to higher DIN export to the coasts, contributing to higher CFs. At the coast, cooler winter temperatures reduce denitrification rates, leading to higher CFs particularly in lower latitudes. My CFs tend to be lower than those of the most advanced method, meaning that the latter might be overestimating impacts. Further major work is the introduction of airborne emissions and transport pathways from air to coasts. My CFs allow as of now for the integration in decision making of the timing of application of fertilisers and of any other DIN emission source to reduce marine eutrophication impacts.

#### **5.05.P-Mo479 REsource Services Depletion Assessment (RESEDA): a parametrized life cycle impact assessment method of dissipative flows**

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Abiotic resources provide multiple services (e.g. mobility, conditioned living space) to society through their use in multiples products (e.g. vehicles, buildings). However, there are some problematic losses of abiotic resources that threaten the provision of those services. These are called dissipative flows, which ends in stocks much more difficult to access than the ones they were initially extracted from. We present RESEDA (Resource Services Depletion Assessment), a parametrized life cycle impact assessment model that quantify the additional efforts for society to meet demand of resource services to compensate dissipative flows. Its objective function is to minimize the cost associated with the extraction of resources. One of its constraints is to meet the demand of services such as mobility, worldwide instantaneous communication or conditioned living space. The demand of services can differ from one scenario to another, therefore we run RESEDA for three different scenarios of demand. It regards (i) the Decent Living Standard, (ii) a business-as-usual scenario on the demand side following production pathways towards Net Zero by the International Energy Agency and (iii) Shared Socio-Economic Pathway 2. At each time step, RESEDA derives the flows of resources from extraction to fabrication of materials to in-use stocks and then to recycling. Dissipative flows are computed considering losses occurring at each life cycle stage. For each scenario, RESEDA is run once with dissipation and then without dissipation. The difference between the two resulting cost for society at a given time horizon enables to determine the marginal cost for society due to the dissipation of a kg of resource (e.g. copper, zinc). It integrates the evolution of future demand, substitutability between functionally equivalent resources, global processing capacity and byproduct production linked to host metals. We developed scenario-based set of characterization factors for 64 abiotic resources. We will present the governing equations of the model as well as the three sets of characterization factors of abiotic resources including both fossils, metals and non-metallic mineral resource such as phosphate.

#### **5.05.P-Mo480 Characterizing Human health Impacts of physical activity in sport and transportation LCAs**

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Physical activity is an important component to consider within various lifestyle determinant (PA, nutrition) when studying the life cycle environmental and human health impacts of various activities such as transportation or sport activities. However, direct use phase impact are presently not included, thus the need to provide factors characterizing the benefit of such activities per km or per hours of different PA types. This presentation aims to address this need by focusing on the following objectives and using the following approach: 1) develop a framework and common parameterization of available dose-response relationships between relative risks of health outcome incidence and physical activity intensities; 2) map dose-response data disease categories to Global Burden of Disease (GBD) background morbidity and mortality rates; 3) compare weighted average health outcomes of PA according to individual disease; and 4) extend the findings to 825 physical activity categories to obtain the hours of healthy life gained per hour of activity as a function of the activity intensity.

Using a meta-analysis of physical activity dose-response data and harmonized relationships for 22 disease and mortality outcomes, the human health impacts of physical activity were expressed as the product of the physical activity level expressed in marginal metabolic equivalent of task (MET) - and a characterization factors in DALY or hours of healthy life saved per MET-hour of activity. Impacts of physical activity on health is substantial, reaching 7 hours of healthy life gained at 10 MET-h/Week, and up to 10 hours of healthy life gained at 40 MET-h/week. 'Coronary Heart Disease Fatal & Non-Fatal'; 'Stroke

Fatal & Non-Fatal'; and 'Diabetes Fatal & Non-Fatal' see the greatest reduction in DALY with higher quantities of physical activity weekly. Starting at 7h at 10 MET-h/Week, reaching as high as 10 h at 40 MET-h/week. For transport related LCA, health benefits per km amounts to between 0.1 and 0.4 hour gained per km biked, and to between 0.25 and 1.1 hour of healthy life gained per hour walking or running. These benefits are up to 20 time higher than the absolute value of the life cycle impacts on human health of around 7E-3 hour lost per km driven in a gasoline car. This study confirms the importance to consider the impacts of physical activity when performing the life cycle assessment of sports activities or transportation over limited distances that involve physical activities.

#### **5.05.P-Mo481 Spatially and taxonomically explicit characterisation factors for greenhouse gas emission impacts on biodiversity**

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In life-cycle impact assessment, currently available characterisation factors (CF) for climate change impacts on biodiversity are highly simplified and do not consider spatial and taxonomic differentiation of species or local climate variability. We develop the first spatially and taxonomically specific CFs for the impacts of 20 GHGs on biodiversity considering 26,648 species across terrestrial and marine ecosystems. Generally, CFs are higher in the tropics, and marine species are affected more severely than terrestrial ones. When global GHG emissions from 2020 are assessed in a scenario with a global temperature rise of 3 °C by 2100, an average of 0.25%, 0.15% and 0.03% of species are negatively affected in 2100 from CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O emissions, respectively, across the globe. The new CFs can be used at different levels of spatial and taxonomic aggregation to quantify co-benefits for biodiversity of climate change mitigation in tools such as life-cycle assessment, input-output analyses, or integrated assessment models.

#### **5.05.P-Mo482 LCA for financial institution's investment and loan portfolio -Biodiversity Damage Assessment Using LIME3-**

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In March 2022, the importance of nature conservation was globally recognized, and nature-related financial disclosure was made mandatory; the GBF was agreed at COP15, requiring financial institutions to disclose natural capital and biodiversity risks. In this study, we analyzed the FY2021 portfolio of the The Norinchukin Bank using Japan's LIME3, utilizing EORA to calculate the amount of activity, considering economic spillover effects by country and region. LIME3 is then applied to quantitatively analyze the biodiversity damage assessment. In the portfolio assessment, the amount of activities such as GHG emissions and land use were obtained, and biodiversity damage (EINES) was evaluated. A detailed analysis of the results by country and industry was conducted. The contribution and impact of specific financial institutions is clearly indicated. An assessment of the environmental impact of financial institutions' portfolio data using LIME3 was conducted. In the future, detailed calculation of activity volumes and analysis by industry will be pursued, with the aim of improving the consistency of the methodology and contributing to the formulation of future regulations.

#### **5.05.P-Mo483 A Highly Regionalized Biodiversity Economic Value Database for Life Cycle Impact Assessment**

*David Font Vivanco, Eco Intelligent Growth, Spain*

Genetic, species, and ecosystem biodiversity provide critical provisioning, regulating, and cultural services to humanity, yet the planet has lost 58% of its biodiversity since 1970 and it is estimated that up to 30% of existing species will be extinct by 2050 with current consumption and production patterns. To reverse this critical trend, it has been argued that the economic valuation of biodiversity can be fundamental for its sustainable use and conservation. While biodiversity is rather unevenly distributed across the world, existing valuation methods are generally context-specific or offer very poor detail, which can lead to sub-optimal decision-making. To address this challenge, we have developed a novel economic valuation method and a database which retains spatial detail to the extent possible. The method departs from the native potentially disappeared fraction of species (PDF)-based characterization factors by elementary flow provided by the Impact World + 2.0.1 database (IW+), with an up to 0.5 x 0.5 degree grid cell resolution for some impact categories. To assign an inclusive economic value to each of these factors, we use an approach based on the total economic value (TEV) of ecosystem services, which covers both use and non-use values. This approach is based on the Ecosystem Services Valuation Database (ESVD), which contains 9,500 value records, as monetary unit per area, from over 1100 studies distributed across all biomes, ecosystem services and geographic regions. Specifically, it is assumed that the future TEV of a given area for a given biome and region is a function of a given additional impact expressed as PDF. Using the land occupation impact category as an example, eliminating all secondary vegetation from an area of a Tropical and Subtropical Dry Broadleaf Forests biome would decrease the value of such area by 22% according to IW+, and so on for the rest of elementary flows until all its value is lost. To account for the elementary flow carrying capacity of each area (e.g., area of secondary vegetation), various additional sources are used, such as global vegetation maps. Uncertainty is comprehensively considered by accounting for different types of uncertainty, such as alternative TEV-PDF functions (e.g., linear and exponential) and economic valuation methods (e.g., damage cost avoided and restoration cost). This database could help practitioners to adequately value biodiversity impacts during the weighting step.

### **5.05.P-Mo484 A Landscape-scale Biodiversity Impacts Analysis of Côte d'Ivoire's Cocoa Cultivation Along Export Supply Chains**

*Shuntian Wang and Stephan Pfister, ETH Zurich, Switzerland*

Agricultural land use for export commodities leads to significant biodiversity impacts. Enhancing transparency in these commodities' production and supply chains is crucial for assessing and implementing effective mitigation policies. Using cocoa cultivation and exports in Côte d'Ivoire as an example, we present a novel framework that combines earth observations, enhanced landscape-scale biodiversity models, and sub-national export supply chains to track the land-use-related biodiversity impacts. Our findings indicate that cocoa cultivation accounts for ~13% of the country's land-use-related biodiversity impacts, with ~87% attributable to cocoa exports. The top ten importing countries account for ~84% of these impacts. Our method offers improved spatial detail compared to existing approaches, facilitating the identification of biodiversity hotspots. Furthermore, we discovered that the land-use-related biodiversity impacts of agroforestry cocoa cultivation are not consistently lower than full-sun cocoa, largely depending on the region's contextual biodiversity importance. Finally, we identified a disparity in biodiversity impacts between global and local perspectives: cocoa cultivation in departments like Guiglo can account for up to 43% of local impact share, while other departments are more important from a global impact perspective. Our transferable framework provides a comprehensive understanding of biodiversity footprint and promotes informed decision-making for sustainable agricultural production, trade, and processing.

### **5.05.P-Mo485 How to better reflect biodiversity impacts within the Environmental Footprint method? A comparison analysis following a consultation approach**

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Biodiversity plays a fundamental ecological role in important economic sectors such as agriculture, forestry and fisheries requiring thus comprehensive attention. The Environmental Footprint (EF) framework includes impact categories associated with biodiversity loss, such as climate change, land use, water use, acidification, eutrophication, ozone depletion, photo-oxidant creation, and ecotoxicity. The IPBES has identified several key drivers of biodiversity loss which are Land Use Change, Direct Exploitation of Organisms, Climate Change, Pollution, and Invasive Alien Species. These drivers frequently intersect and their effects can accumulate, resulting in intricate and extensive ramifications for biodiversity and ecosystem services. Additionally, the impacts of different land management approaches remain insufficiently differentiated and not capturing all aspects. It is noteworthy that land use impact categories currently embedded in the EF framework predominantly concentrate on soil quality and fail to encompass a wider diversity of land use approaches required for a comprehensive assessment of biodiversity, particularly in terms of land management practices. The primary objective of this work was to delineate previously unaddressed impacts on biodiversity as a distinct impact category within the EF. Additionally, it involves revisiting the relevant impact categories in the EF, particularly those associated with land use. This evaluation also seeks to explore the medium- and long-term requirements for other methods, including climate change, ecotoxicity, eutrophication, and others. The outcome could be either a differentiation and complementing of the current EF LCIA framework (e.g. by separating out a biodiversity sub-indicator composed of biodiversity-related currently covered midpoints plus additions of yet unconsidered ones) or a complete further development, as main but not only potential solutions. The proposed method will be interim, and the EF group will keep following the advancements of several platforms.

### **5.05.P-Mo486 Land-Use-Based Biodiversity Impact Assessment Methods in food LCA**

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Agricultural land use presents a substantial threat to global biodiversity. To comprehensively evaluate the biodiversity impacts of food, it is imperative to systematically incorporate the country of origin for each product. Beyond the production site, the quantities of consumed products also hold relevance in the assessment of food-related impacts. Therefore, diet level assessments are ideal when identifying hotspots and finding measures to decrease the biodiversity impact of food consumption.

In this study, we employed two recent land-use-based biodiversity life cycle impact assessment methods (Chaudhary and Books, 2018; Kuipers et al., 2021) to assess the biodiversity impacts of five dietary scenarios, including the current Finnish diet and four alternatives gradually reducing animal-origin food intake. The assessment utilized the previously developed FoodMin model, which evaluates climate impact and nutritional quality of diets based on 90 food product groups. For the biodiversity impact assessment, the countries of origin and the land use, as well as land-use changes, associated with each product category in those countries were assessed utilizing national import data and international statistics on yield and land-use change.

The study revealed substantial variability in biodiversity impacts, with a 60-fold difference in magnitude depending on the assessment method. Land-use change, particularly associated with feed production, emerged as a primary driver of impacts, reducing the dietary impacts with the intake of animal-origin foods. At the same time, particularly for beverages, sugars, and sweets, occupation impacts were higher than the impacts originating from land-use change.

An analysis of the two assessment methods revealed that absolute comparisons of results obtained with these methods are inappropriate. On a product-level basis, also different relative results were obtained, influencing which products appeared most burdensome.

The results emphasize the critical importance of tracking the production locations of each product in biodiversity impact assessment, and the necessity of considering both land-use change and occupation impacts. Future research should focus on developing biodiversity impact assessment methods, especially on addressing impacts on a local scale, improving land-use change methodology accuracy, and analysing the impacts of diverse agricultural management practices.

#### **5.05.P-Mo487 Sustainability in Wheat Farming: A Critical Examination of Fungicide Practices in Belgium Through Life Cycle Assessment.**

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Fungicide treatments represent a critical concern in Belgian wheat farming due to the prevalence of diseases such as fusarium, septoria, and yellow rust, which exert significant pressure on yields. The optimization of fungicide programs is essential, requiring the strategic use of multiple substances with distinct modes of action to mitigate resistance development. This challenge is further exacerbated by regular adjustments in fungicides regulations and the introduction of new compounds.

Despite the crucial role of fungicides in agriculture, there is a notable scarcity of Life Cycle Assessment (LCA) studies addressing their environmental impacts. However, decision support systems for fungicides application already exist, and integrating LCA into these tools could provide an additional valuable criterion for decision-making, promoting more sustainable agricultural practices. This research aims to address key challenges surrounding the environmental impacts evaluation of fungicides use, focusing on wheat production in Belgium. These challenges encompass limitations of field emissions modelling in current Life Cycle Inventory (LCI) databases and the assessment of potential toxic effects on human health resulting from the consumption of residues on plants.

In this context, the framework developed by the OLCA-Pest project is applied in order to model field emissions of fungicides and estimate the characterization factors for human toxicity associated with the fungicidal substances currently employed in Belgian wheat farming. This preliminary analysis focuses on a specific two-step program, involving triazole, SDHI, and multi-site fungicides. Initial results reveal significant environmental impacts on toxicity categories, with notable variations across different substances. The outcomes of this analysis will serve as a benchmark for the LCA of alternative fungicide programs, including innovative sustainable pest control solutions currently under development within a project funded by Belgian public authorities.

This approach aims to provide valuable insights into the environmental and human health impacts of fungicide programs in Belgian wheat farming. Integrating such LCA studies into local decision support tools holds promise for refining fungicides use strategies, ultimately contributing to a more sustainable and resilient agricultural sector in Belgium.

#### **5.05.P-Mo488 Environmental Assessment of Bio-based Fertilizers: adaptability of the non-LCA methods into LCA**

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Bio-based fertilizers (BBFs) are crucial for reducing dependency on mineral fertilizers and developing a circular economy. Life Cycle Assessment (LCA) is the primary tool for measuring BBFs environmental impacts. However, no specific adapted methods exist to evaluate some key impacts such as biodiversity, carbon sequestration, or pathogens presence. Thus, the incorporation of complementary models (non-LCA) can lead to the development of a comprehensive evaluation with more robustness that can mitigate the main challenges of representativity, regionalization, and modeling in the LCA framework.

This work aims to identify the most cited environmental burdens caused by BBFs, the methods the scientific community has used to assess them, and their possible coupling with the LCA framework. A preliminary screening to identify BBFs main environmental concerns was conducted using a two-step literature review. A comprehensive search was conducted to review the available modeling, tools, indicators, and methods. Then, we collected the most representative case studies in which these models were applied coupled with the LCA approach. We developed an integral analysis of the strengths and weaknesses of each one of them.

Our results indicate that the most critical environmental concerns of BBFs were the effects on soil properties, the soil organic carbon dynamics (sequestration/sink), biodiversity, and the presence of heavy metals. For example, the biogenic carbon accounting in LCA uses two possible criteria: i) the uptake-release accounting or ii) the exclusion approach employed in the Product Environmental Footprint methods (PEF). For the biodiversity assessment, a method that covers the drivers causing pressure and includes evaluating ecosystem services along value chains seems the most effective. Also, in this case, a critical evaluation of the differences in the LCA results with midpoint and endpoint indicators is recommended. Finally, risk assessment is the most widespread and commonly used method to evaluate the potential toxicity of heavy metals in human health, crops, or soils.

LCA still has aspects/fields in which further development and research are needed. Still, coupling methodologies outside of the LCA approach can represent advantages to determine and evaluate more precisely the environmental impacts of BBFs,

obtaining robust, reliable, and comparable results. Moreover, they can help minimize the lack of specific standardized LCA guidance.

#### **5.05.P-Mo489 Biogenic Carbon – Challenges For Product Life Cycle Assessment and Opportunities for Streamlined Carbon Tracking**

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Chemical manufacturers are utilising life cycle assessment to report the environmental impacts of their products. These are usually cradle-to-gate assessments under methodologies such as Together For Sustainability (ISO14067). One challenge for manufacturers performing assessments to ISO14067, is applying a -1/1 methodology to carbon flows. Under this methodology, CO<sub>2</sub> sequestered in a product is characterised as -1 kgCO<sub>2</sub>e/kg, while biogenic CO<sub>2</sub> emissions are characterised as 1 kgCO<sub>2</sub>e/kg. This contrasts with 0/0 methodologies that are utilised in the Product Environmental Footprint method and characterisation models such as IPCC 2021 GWP 100a (excluding uptake) where all biogenic carbon flows are characterised as 0. LCA modelling platforms allow practitioners to switch between -1/1 and 0/0 methods. However, background datasets e.g., ecoinvent, used by platforms apply allocation, which doesn't always align to the physical allocation required for carbon flow modelling.

We have observed that when applying a -1/1 calculation method, individual ecoinvent processes' biogenic emissions do not balance with their carbon uptake. While this is *possible*, it is improbable, unless the carbon is released in the form of CH<sub>4</sub>, rather than CO<sub>2</sub>. Likewise, we observed that some processes result in carbon uptake so much higher than biogenic emissions, that the carbon content of the product would have to be in excess of 100%. This could lead to practitioners drawing inaccurate conclusions particularly when undertaking comparative LCA.

We have sought to address this by creating a calculation to characterise and balance carbon flows. We tested this calculation on 26 food datasets and observed that at the extreme, datasets possessed up to five times as much carbon uptake as biogenic emissions.

Through our assessment we were able to identify where the imbalance occurred and automatically propose a correction. This calculation could be applied to any ecoinvent material process and would enable users to report GWP impacts under a -1/1 method that balance carbon flows. This should help avoid inaccurate assessments under systems like ISO14067.

#### **5.05.P-Mo490 ProScale-E - an easy-to-use scoring approach for ecotoxicity potential assessment.**

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This abstract presents ProScale-E, a hazard classification based scoring approach for ecotoxicity potential assessment suitable for, for example, LCA in the SSbD context in early innovation phases. The ambition for the current ProScale-E model was to a) obtain a similar hazard banding model as in ProScale-H, for substances classified according to ecotoxicity (hazard classes in the H400 family) thereby accounting for the severity of impacts, b) accounting for potency of substances, and c) facilitating inclusion of release, fate and degradation of substances. A task force consisting of experts from member companies of the ProScale consortium has worked through a series of meetings organised with 2-3 weeks interval and with various aspects of the model elaborated in between these meetings. During this time period, the Task Force representatives have discussed with internal experts in order to come up with an agreeable classification and scoring scheme. The topics covered were divided into three factors: 1) environmental release, 2) environmental transformation and fate, 3) environmental hazard. A preliminary scoring system for eco-toxicity hazards based on a grouping of hazard statements from CLP is now in place. Comparing with the hazard banding model of the suggested SSbD framework, there are similarities between the displayed ProScale-E scoring model and the Levels as suggested in the SSbD framework, but there are also important differences. In particular the introduction of significance to the M-factor is a key aspect of ProScale-E, as the M-factor is based on EC50 values, and therefore introduces the potency of a substance into the scoring.

#### **5.05.P-Mo491 Methods to Determine Freshwater Aquatic Toxicity Effects Factors and Their Influence on Product Environmental Footprinting**

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The potential for a chemical substance to elicit adverse effects to aquatic ecosystems can be represented by a single toxic potency value. The value is derived from the database of controlled, empirical aquatic toxicity studies and sometimes from predictive models. The distillation of numerous study and model results of varying quality for a broad range of standard and non-standard test species to a single value is scientifically challenging. To support consistent and reproducible potency estimates, researchers and regulators have undertaken numerous efforts to create regimented systems for translating aquatic toxicity databases to single potency values. In product environmental footprinting (PEF) for environmental sustainability assessment, the consensus method is to determine the hazardous concentration to the most sensitive 20% of aquatic species tested (HC20) using a probabilistic model that simultaneously accounts for all valid study results – a species sensitivity distribution (SSD). These HC20 values are a critical element of the freshwater ecotoxicity characterization factor used to compare among alternatives for PEF. Regulatory environmental risk assessment uses a similar approach. Here, SSDs are used,

but with prescriptive guidelines, varying by jurisdiction, that require an extensive minimum data set in terms of study quality and breadth of species diversity. The HC5 is generally used as an absolute level of environmental protection desired. However, because of the stringent data requirements for SSDs in regulatory settings, it is more common for potency to be characterized using a single test result associated with the most sensitive species/endpoint tested modified by an “assessment factor” to account for uncertainty. This presentation explores the implications of solely using SSDs in PEF, regardless of database robustness. First, we simulate the variability expected among data-poor SSDs by using subsets of data from data-rich substances to calculate “pseudo-data-poor” SSDs and compare the results. Second, these results are compared using the most sensitive species test result according to regulatory guidelines as an alternative estimate of potency. The pragmatic and scientific pros and cons of each method and recommendations for appropriate use cases are presented.

#### **5.05.P-Mo493 Including non-linearity in life cycle impact assessment: a case for GWP and energy transition scenarios**

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Life cycle impact assessment relies on fixed characterization factors. These fixed factors implicitly assume a constant relationship between biosphere flows, the condition of the receiving Earth compartment, and the resulting impact. However, environmental impacts depend on both the pressure and the state of the environment, implying that fixed characterization factors may be misleading in the calculation of impacts and the activities they inform.

A notable example of adaptation to changes in compartments is the Global Warming Potential (GWP) indicator. For certain emissions, GWP characterization factors have been adjusted; for instance, methane, which was once considered 25 times more impactful than CO<sub>2</sub>, is now considered 28 times more impactful. These adjustments are contingent upon the concentration of greenhouse gases in the atmosphere. The higher the concentration, the greater the relative impact.

Life Cycle Assessment (LCA) is frequently employed to evaluate the impact of technological changes in future renewable energy scenarios. However, the characterization factors used for scenario assessments in years 2050 or 2030 often do not account for anticipated changes in the atmospheric concentration of various gases. These concentrations are typically estimated using climate models.

In this study, we propose a dynamic evolution of GWP characterization factors aligned with IPCC scenarios. We used PREMISE to calculate structural changes in the Ecoinvent inventories based on different scenarios and integrated assessment models. Subsequently, we developed a dynamic LCIA method within Brightway2 and integrated it into the ENBIOS tool. We conducted an ENBIOS assessment for various energy transition scenarios in Europe.

Our findings support the notion that estimated impacts will be higher than those derived from a linear definition of GWP as shown in the figures below. As these changes are currently not considered in energy modeling, the prevailing designs for energy pathways may not be the most suitable options for achieving a truly sustainable energy transition.

#### **5.05.P-Mo494 Life Cycle Impact Assessment with ReCiPe**

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ReCiPe is a multi-impact life cycle impact assessment method available for practitioners. It is a comprehensive LCIA methodology, which provides a harmonised implementation of cause-effect pathways for the calculation of both midpoint (18 categories) and endpoint (3 areas of protection) characterization factors (CFs). To our knowledge, it is one out of only two methods that are currently available that provide both mid- and endpoint factors. Another unique feature is that in ReCiPe, different value choices were grouped into a limited number of scenarios or perspectives. The individualist perspective is based on short-term interest, impact types that are undisputed, and technological optimism regarding human adaptation. The hierarchist perspective is based on scientific consensus regarding the time frame and plausibility of impact mechanisms. The egalitarian perspective is the most precautionary perspective, considering the longest time frame and all impact pathways for which data are available. Review articles on LCA case studies show that ReCiPe has been one of the most used LCIA methods since its release in 2008.

Despite its unique features and high level of use, ReCiPe also has some shortcomings that need to be addressed in a next update. The most important shortcomings are listed below.

For the area of protection ecosystem damage, biodiversity loss is currently determined in different ways, depending on (data available for) the impact category. While for land use local biodiversity loss is quantified, for global warming global biodiversity loss is quantified. Furthermore, some impact pathways are currently not included, such as microplastic impacts on ecotoxicity, and nitrogen impacts on freshwater eutrophication.

For the area of protection human health damage, an important shortcoming is the lack of archetype-specific CFs for particulate matter formation. Research has shown that the emitting sector and region are important factors that determine the magnitude of the CFs, which is not reflected in the currently available country-specific factors.

Finally, to improve applicability and representativeness of the CFs, the factors need to become available for input-output modelling, and a plan to address uncertainty in the CFs is needed.

The goal of this work is therefore to develop new methods to be implemented in a ReCiPe update, to provide the user with a high-level method for future LCAs. In the poster, foreseen updates will be outlined and discussed.

#### **5.05.P-Mo495 Improving substance coverage for more accurate ecotoxicity normalisation factors – A Consortium-based approach**

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Normalisation and weighting are used to aggregate the results of multiple impact categories into a single score in life cycle assessment (LCA), allowing an easier interpretation of the results and empowering the use of LCA in decision making. While some weighting approaches, such as the one recommended by the Product Environmental Footprint (PEF), are based on panels' opinions, the normalisation step is based on empirical data associated with uncertainty and variability. This additional layer of transformation of the results means that the uncertainty of a single score is increased by the uncertainty of normalisation factors. It is therefore crucial to continue working towards improving normalisation factors.

Specifically, normalisation factors of toxicity/ecotoxicity impact categories are known to be particularly uncertain, due to the inherent complexity of these impacts: as any substance can be associated with ecotoxicity/toxicity impacts, the number of substances to be characterised and included in these methods is enormous, which makes the collection of inventories to build normalisation factors especially challenging. In the EF 3.0 method package, the three USEtox impact categories were given the lowest grade of III for both 'Inventory coverage completeness' and 'Inventory robustness' by the European Commission. This is explained partly by the fact that some sectors are not covered at all by these inventories, such as the cosmetic sector for which close to no ingredients are included.

In this work, we investigate the magnitude of inaccuracy of the ecotoxicity normalisation factor (EF method) and present ways to improve it for the cosmetic sector. We took advantage of the EcoBeautyScore Consortium to gather data about global usage of beauty and personal care products across many companies and deduced missing inventories of substances emitted to the environment related to that sector. We report how to improve normalisation factors more generally, draw recommendations for future practitioners and illustrate the benefits of data-driven, industry-wide collaborations.

#### **5.05.P-Mo496 Earth System Functioning as a Separate Area of Protection for Life Cycle Impact Assessment**

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The impact categories in Life Cycle Assessment (LCA) are developed to capture potential environmental impacts, on midpoint, endpoint, or damage level. For several decades now, the considered impact pathways predominantly aim for one of the three principal Areas of Protection (AoP): Human health, Ecosystem quality, and Resources. These AoPs determine which elementary flows are considered, which environmental processes are included, and how the impacts are evaluated. Conversely, the flows, processes, and impacts not directly relevant to the AoP are not considered. While the three AoPs dominate, others have been proposed over the years, for example ecosystem services. Yet another environmental issue and another dimension of sustainability research is related to Earth System (ES) functioning. According to the field of Earth System Science, planet Earth can be viewed as one complex adaptive system which provides the unique conditions which allow flourishing of life on Earth. In the geological epoch of the Anthropocene, human activity now competes with natural regulatory mechanisms and affects the stability and functioning of the ES. I propose ES functioning as additional unique AoP to capture this crucial area of environmental science. ES functioning is inherently intertwined with the other AoPs. From one perspective ES stability and functioning can be seen as a vital precondition to human health, ecosystem quality, and resource provisioning, hence a midpoint in those impact pathway. But at the same time ecosystems and humans are inherent parts of the ES and affect its functioning, thus ecosystem quality and human health to a degree could be seen as midpoints to the ES AoP. In the same way, ecosystem quality is necessary for human health. While there are many common elements with the current AoPs, the perspective, and thus the considered impact pathways, would often differ. Specific impact assessment models and midpoint and damage-level impact categories could be developed to capture this AoP. In fact, the process has already started with impact assessment models targeting the Planetary Boundaries framework. Indeed, the issues and formulations presented in the Planetary Boundaries, or Earth System Boundaries, could provide an apt starting point and guidance on which impact pathways should be considered. Establishing ES functioning as a unique AoP would provide a useful addition to the comprehensiveness of LCA studies.

#### **5.05.P-Mo497 Choices Mechanism in Models Construction for Life Cycle Impact Assessment of Natural Resources.**

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Life Cycle Assessment (LCA) is a widely used tool for assessing the environmental impact of products and processes. Like any other decision-support tool, LCA characterization models contain numerous so-called value judgments (whenever the modeler makes a subjective choice between two modeling options that cannot be objectively distinguished). The presence of subjective choices in LCA has been a source of controversy within the LCA community, but the debate remains unresolved. The assessment of natural resource depletion in LCA is a typical example of this controversy: there is no consensus on what safeguards subjects should be assessed through the impact category, and there are over 27 methods for assessing impacts in the "natural resources" category, each quantifying different elements. These variations produce differences in results that can influence decision-making. We need to understand modelers' decisions and model construction in LCA. This research aims to identify the various factors that influence modelers when building a new model, the contextual, epistemic, and personal influences leading to choices.

The methodology of this research combines a critical literature review of existing natural resource characterization methods with qualitative interviews and content analysis, aiming to identify the factors influencing modelers and understand the choice-making mechanisms inherent in the construction of life cycle impact assessment models for natural resources.

For now, 43 motivations were identified, classified in 8 categories such as pragmatism, feasibility, acceptability, LCA culture, modeling technique, colleagues or hierarchy influences, personal experience, literature, and data. Certain trends have been analyzed in greater detail, to show how the different motivations for a decision can interact.

The study found that modeling tends to be a collaborative effort. The study examined motivations for modelers' choices, driven by tools and team expertise, contextual values, like feasibility, available data, and acceptability. We conclude that models are not purely objective tools, but social constructs influenced by decisions meaning model results depend on the context, leading to the need for transparency in modeling procedures.

#### **5.05.P-Mo498 Substitution in Circular Footprint Formula: feasibility showcase through nutrient recycling to secondary fertilizer**

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In response to heightened sustainability awareness, regulations promoting resource recycling are exemplified by initiatives like nutrient recycling from wastewater/waste to produce secondary fertilizer. LCA has been applied in such cases to support decision-making.

Understanding various approaches that address multifunctionality, an inherent facet of recycling in end-of-life scenarios, is imperative, as it induces alterations in results and assigns different incentives. Circular footprint formula (CFF) is proposed as an alternative for end-of-life modeling, where avoided burden of primary material production combined with a Quality factor (Q), a typical approach often associated with consequential LCA (CLCA) approach, is presented. Yet, the applicability of CFF in the unique context of nutrient recycling to secondary fertilizers remains uncertain, as their composite nature entails nuanced quality and function-related properties. Only through a granular assessment at the nutrient level can these properties be adequately captured. Additionally, official documents failed to define "quality" as this term embodies a feature: despite the product can fulfil the same function, it can exhibit distinctions in emissions in later life phases.

A case study is presently underway to investigate the feasibility of CFF in terms of substitution and substitutability through Q factor. In total, six scenarios are designed in both attributional and consequential systems to demonstrate applications of 'standard' substitution and facilitate a comparative analysis with the CFF approach. Within this schema, considerations pertaining to the functional unit and system boundary will be expounded. Both well-established and emerging technologies with a production of organic and mineral secondary fertilizers are assessed. Both types of secondary fertilizers will offer distinct aspects in terms of Q factor and substitutability.

The objectives of this presentation are (i) to illustrate disparities between "standard" substitution and CFF in a specific case of secondary fertilizer production, (ii) to identify imperative parameters when calculating substitutability, and (iii) to examine the applicability of CFF. The results outlined in this presentation call upon researchers to dedicate increased focus to exceptional cases, fostering advancements in both CFF and substitution approaches.

#### **5.05.P-Mo499 Comparing approaches for assessing Absolute Environmental Sustainability: a case study on the EU Consumption Footprint**

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Absolute environmental sustainability assessment (AESAs) is an emerging approach that enables identifying those systems that are inherently environmentally sustainable by combining Life Cycle Assessment (LCA) with absolute limits, most commonly represented by the Planetary Boundaries (PBs) framework. The ability to assess the absolute environmental sustainability of a

product system is paramount because although we are improving the environmental performance of individual products (i.e. the eco-efficiency), the total environmental impacts of our society continue increasing due to growing population and per capita consumption. For this reason, AESA is attracting increasing attention in academia and beyond, including for policy-making.

One potential obstacle to the widespread uptake of AESA is the lack of a consensus methodology. In this work we focus on two key methodological aspects: i) how to harmonize LCA indicators with the Planetary Boundaries control variables, and ii) how to allocate the Safe Operating Space delineated by the Planetary Boundaries to individual systems. We compare existing literature approaches and investigate their qualitative and quantitative differences, using as a case study the EU Consumption Footprint developed by the Joint Research Center (JRC). The harmonization aspect is investigated via 3 approaches that address different steps of the life cycle impact assessment stage. The allocation aspect is explored via 4 alternative approaches, representing those that are most commonly adopted. The results of our study will enable developing recommendations for the application of AESA and potentially for the establishment of a consensus methodology.

#### **5.05.P-Mo500 Limitations of the resource depletion assessment in the energy transition**

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There is a clear nexus between decarbonisation and resource consumption in the energy transition. However, while the methods to measure the climate change are well assessed, the way to assess resource depletion is still ongoing. The gaps are both due to Life Cycle Inventory limitations and LCIA methods. This study shows the variability in the results obtained across years, using one of the most common LCA database and the impact assessment methods suggested by the European Commission, and applying them to the Italian energy scenario at 2030. The analysis of the Italian Energy scenario at 2030 was performed in different years, following the evolving decarbonisation goals in the energy sector. Meanwhile also the database used in the background changed (from Ecoinvent 2.2 to Ecoinvent 3.9.1) and the assessment method recommended by the European Commission moved from ILCD to EF. Even though the trade-off between decarbonisation and resource depletion was confirmed across the years, the variability among the depletion results were notable and not explainable with the differences in the energy scenarios. Analysing the changes in the results across different ecoinvent versions and LCIA methods, this study investigated the reasons behind this variability. On the inventory side, an intrinsic multi output sector such as the mineral one with coproducts originating from different processes across the production chain, makes it difficult to solve multifunctionalities with the classic allocation methods; this is particularly true for mineral resource flows. In the evolution of Ecoinvent database different options have been put in place to solve this problem: from resource correction factors (Ecoinvent 2.2) factor to resource flows entering the technosphere lower along the production chain instead of entering the extraction process (Ecoinvent 3.3). In the current version of the database (Ecoinvent 3.9.1) there is a pure economic allocation, and resource consumption does not reflect the mineral and metal content in the final product. If this allocation might be coherent with an intertwined sector such as the extraction sector, this economic aspect should be reflected also in the impact assessment method that evaluates the depletion of resources. For short-medium term goals such as the decarbonisation of the energy system, our suggestion is to use prices of minerals and metals as proxy of their availability.

#### **5.05.PC Life Cycle Impact Assessment – Advances in Modelling and Application**

#### **5.06 Navigating the Complexity of Plastic Life Cycles: Interdisciplinary Challenges and Advances in Assessing Environmental Impact**

##### **5.06.T-01 A Meta-Analysis of LCA Studies and Global Warming Potential on Polylactic Acid**

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Bio-based polymers are often perceived as a solution for sustainable plastic manufacturing. Frequently cited advantages include their potential lower carbon footprint compared to fossil-based polymers and alternative end-of-life pathways in case they are also biodegradable. This also applies to polylactic acid (PLA), one of the most advanced biopolymers on the market. Over 80 life cycle assessment and carbon footprinting studies of PLA have been performed since the early 2000s, with many including a comparison to fossil-based plastics. In this review, the results from these studies were collected and compared to clarify, whether an overall conclusion can be made about the advantages of replacing fossil-based plastics with PLA. The main focus was placed on global warming potential. When looking at cradle-to-gate (resin manufacture) scope, the median value for PLA was 1.63 kg CO<sub>2</sub> eq/kg PLA, which is indeed lower than the fossil-based polymers such as PET, PP, or PE. However, when the median values from cradle-to-grave studies were weighed up, PLA with a median value of 3.91 kg CO<sub>2</sub> eq/kg showed comparable results to the investigated fossil-based polymers. This discrepancy in the conclusions between the scopes is primarily a result of biogenic carbon accounting, where the cradle-to-gate results benefit from the CO<sub>2</sub> uptake by feedstock. A great variation was observed in the results for PLA between the different studies, stemming from the complexity of the LCA and the different choices made by the authors. Those include type of feedstock, electricity mix, end-of-life option, or aforementioned biogenic carbon accounting. It was noted that especially moving towards using renewable energy is beneficial in terms of reducing global warming potential.

#### **5.06.T-02 A Life cycle Assessment with microplastic aquatic ecotoxicological impacts included.**

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The environmental impacts from plastic pollution are typically not included in Life Cycle Assessment (LCA). As a result, an LCA performed from plastic products do not reflect the full impact spectrum of the product. In this study, characterisation factors (CFs) were developed for macroplastic (MaP, >5 cm) and microplastic (MiP, <5 cm) emissions related to freshwater ecotoxicity and marine ecotoxicity. These CFs were added to the ReCiPe2016 impact assessment method. To obtain the CF, environmental fate and ecological effects of MiPs were assessed. The environmental fragmentation of MaP to MiP were accounted for through a conversion factor. The developed CFs were applied to a case study LCA, which includes two consumer films, made from 3 different polymer types (PP, LDPE, PET). Mass loss estimates of MaP and MiP per functional unit were accounted for through adaptations of an existing plastic litter material flow analysis model (Schwarz et al., 2023). The CFs were obtained for the three polymers by assessing the Effect and Fate Factor. Effect Factor was kept constant for all three polymers and based on literature research. Fate Factor modelling was executed using Simplebox4plastics (Quick et al., 2023) for the three selected polymers. Incorporation of MiP in the case studies resulted in significantly higher ecotoxicity midpoint impacts, maximally >99% increase of impact, depending on ReCiPe2016s cultural perspective and polymer type. The endpoint indicator on ecosystem quality resulted in a limited increase, with a maximum increase of 3.3%. The results indicate the relevance of including plastic pollution in ecotoxicity impact indicators. The degradation rate of MiP played a key role in fate modelling. Improvement of Effect and Fate factors used to obtain the CFs is recommended for future research.

#### **5.06.T-03 Modeling Environmental Impacts of Plastics in Life Cycle Assessment: Effects of Biodegradation**

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Plastic emissions to the environment and the rapid accumulation of plastic particles in different environmental compartments are posing a threat to ecosystem quality and human health. To determine the magnitude of their potential damage within the full life cycle of a product, it is necessary to include the quantification of potential impacts from plastic emissions in Life Cycle Assessment (LCA) modelling. To integrate plastic emissions into LCA, characterization factors are needed that commonly consist of three elements: a fate factor, an exposure factor, and an effect factor. In this context, fate factors quantify the distribution and longevity of plastics in the environment. However, research on these fate factors is still limited, especially for biodegradable polymers. Hence, the main objective of this research is to determine the fate factors of biodegradable polymers (polylactic acid, polybutylene succinate-co-butylene adipate, polycaprolactone) based on primary experimental data for the marine environment. In former research, fate factors were based on literature data, assuming that the degradation kinetics of macroplastics and microplastic particles would be similar. This work tests this assumption by comparing the degradation kinetics of macro- and microplastics under the same conditions. Although polymers are commercially used in different grades, most degradation data found in literature were assessed for pure polymers. In this work, the degradation kinetics of different grades of a commercial plastic (differing in molecular weight) are compared to explore whether this simplification is suitable. Furthermore, in this work, degradation at the ocean average temperature (4 °C) is compared to degradation at elevated temperatures (20 °C), as the latter conditions are often used in the literature on polymer degradation. The degradation data are obtained by monitoring the oxygen consumption, pH, and microbial growth over a period of six months in natural seawater, sampled at the coast of Oostend (Belgium). The determined degradation rates are combined with sedimentation rates to obtain fate factors, based on USEtox recommendations. These fate factors are subsequently integrated into LCA characterization factors. Resulting characterization factors are tested in an LCA case study of a synthetic sports shirt, allowing for an assessment of the relative importance of microplastic impacts compared to the rest of the life cycle.

#### **5.06.T-04 Environmental assessment of emerging bio-based polymers: Integration of ex-ante and prospective LCA**

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Biomass is a promising renewable feedstock to defossilize the chemical sector, transitioning from a fossil linear economy to a circular bioeconomy. However, this transition has multiple challenges. The sustainability of bio-based technologies depends on biomass availability at lower risks of impacts to food security and nature conservation, and many of these technologies are still at a low technology readiness level (TRL), thereby hindering a fair benchmark with their fossil counterparts. To assess a more robust comparison of environmental performance of emerging bio-based polymers against their fossil alternatives, this study applies a combined framework integrating ex-ante and prospective life cycle assessment (LCA). The framework includes a stepwise approach to scale-up technology maturity (ex-ante) followed by an integration of the up-scaled inventories with future projections of background data (prospective). We assess the potential for climate change mitigation by substituting fossil polystyrene (PS), polymethyl methacrylate (PMMA), and polycarbonate (PC) produced in Europe (EU) with poly-isosorbide methacrylate (PIMA), an emerging polymer produced from lignocellulosic feedstock (LCF) that is at low TRL. To avoid competition with food production, the assessment explores biomass resource availability in EU to produce PIMA considering country-specific availability of LCF (i.e., agriculture and forest residues) for a sustainable supply potentials (i.e., at levels that soil carbon pools and nutrients are not depleted). In total, all necessary PIMA to replace the fossil polymers can be annually produced, using only 5% of the sustainable available residues in EU. The replacement of the fossil polymers using PIMA impacts at lab-scale results in emissions of 43 million tonnes of CO<sub>2</sub>eq per year, as the lab-scale impacts are higher than the

fossil ones. The ex-ante LCA reduces total impacts by 80%, but this is insufficient to result in net mitigation relative to the fossil polymers. However, when combining ex-ante and prospective LCA, a net climate change mitigation of -7 MtCO<sub>2</sub>eq/yr is achieved (up to -6 kgCO<sub>2</sub>eq/kg polymer when PIMA replaces PC). This is about 12% of emissions from the chemical sector. The integration of ex-ante and prospective LCA has the potential to secure a more consistent assessment of climate change mitigation potentials in the EU chemical sector, and thus better guide research and development investments relative to conventional LCA.

## **5.06.P Navigating the Complexity of Plastic Life Cycles: Interdisciplinary Challenges and Advances in Assessing Environmental Impact**

### **5.06.P-We439 Regionalized sourcing strategies of lignocellulose residues for a net-zero plastics industry**

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Climate change, biodiversity loss, and pollution are the three major environmental challenges that the world is currently facing. The primary cause of climate change is human activities, particularly the increasing use of fossil fuels. The chemical industry is the largest consumer of fossil fuels and the third-largest emitter of greenhouse gases, making it imperative to find sustainable alternatives. Bio-based plastics production is seen by the industry as a lever to reach net-zero greenhouse gas emissions. However, the availability and environmental impacts of biomass vary widely across regions and types, which are often oversimplified or ignored in previous studies. We provide a comprehensive assessment of low-impact lignocellulose residues as plastics feedstocks, considering their spatial distribution, quantity and environmental impacts.

We utilize the Global Biosphere Management Model (GLOBIOM) to project the availability of these residues on the grid-level. We estimate that 2.6–5.2 Gt dry mass of lignocellulose residues will be available in 2050, with Brazil, China, India and the United States accounting for half of the potential supply. Regionalized prospective LCAs are then conducted to demonstrate the future environmental implications of utilizing lignocellulose residues as chemical feedstocks. With the assessment of more than 600 country-feedstock combinations, this study emphasizes the regional difference in impacts related to biomass, especially concerning land use-related impacts. We find that forest residues have 85% lower climate change impacts but triple the land-use related biodiversity loss impacts compared to agricultural residues. The comprehensive LCA database generated in this study is open-sourced to bridge the existing gap between process-based LCAs with limited data coverage and system-wide analyses of the plastics industry that oversimplify biomass-related assumptions. A case study is provided to compare the impacts of the alternative feedstock-based plastics with the alternatives. This study serves as a foundational resource by offering valuable insights into the regional availability of biomass and the associated environmental impacts of its utilization. They play an important role in shaping the future development of global and region-specific, comprehensive net-zero strategies for the plastics industry.

### **5.06.P-We440 Methodology and case studies to address potential impacts of plastic emissions in life cycle assessment**

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Products made of plastic often appear to have lower environmental impacts than alternatives. However, present life cycle assessments (LCA) do not consider possible risks caused by the emission of plastics into the environment. This study presents a new approach to address the environmental impacts of plastic emissions in life cycle assessments (LCA) by proposing characterization factors (CFs) to calculate the impacts of plastic pollution. The CFs expressed in plastic pollution equivalents are based on the residence time of plastics in the environment and take into account the precautionary principle.

The method developed focuses on defining and quantifying plastic emissions in LCA and estimating their fate in the environment based on their persistence. In the fate model, the fate of plastic emissions is influenced by the environmental compartment they are initially emitted to, their redistribution to other compartments, and their degradation speed. The specific surface degradation rate (SSDR), emission shape, and characteristic length of the plastic are key factors affecting its degradation speed. SSDRs were derived from a literature review, and uncertainty assessments were conducted using the pedigree matrix approach. The fate factor (FF), which represents the residence time, is calculated by determining the area below the degradation curve of an emission.

The results of this research include degradation measurements (SSDRs), a surface-driven degradation model, redistribution patterns, FFs based on residence time, and an uncertainty analysis of the proposed FFs. The FF values vary depending on polymer type, size class, shape, and initial compartment, with some values exceeding 1000. Polymer types were grouped into six clusters based on compartment-specific FFs and total compartment-weighted FFs. The proposed FFs can be used as CFs and can be further developed to integrate exposure factors and effect factors related to human or organism exposure and impacts on species.

The developed methodology aims to support product designers in making informed choices regarding materials, specifically plastics, by estimating potential risks associated with plastic emissions.

The method was already applied in various case studies e.g. focussing on PHB products, and different strawberry production methods. The latter case study will also be presented.

#### **5.06.P-We441 Including Microplastics Emissions Impact in Sediments in Life Cycle Impact Assessment**

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Washing and handling of synthetic fibers could be responsible for the release of 500,000 tons of microplastics (MPs) per year into marine ecosystems. When released, MPs first reach the water column where they can be ingested by pelagic species. MPs can then reach marine sediments, a potential sink, where they can affect benthic species. However, current life cycle impact assessment methods do not consider the impact in the sediments of MPs emissions.

This work builds on the MarILCA working group advances by including the *physical effect on biota* of MPs in the sediments sub-compartment. The fate of MPs in the marine environment is quantified by calculating the residence time of MPs in the water column and sediments sub-compartments, of different polymers, sizes and shapes. Further, a combined exposure and effect factor ( $EEF_{\text{sediments}}$ ) for microplastics in sediments is also developed, allowing to obtain updated marine characterization factors (CFs) for MPs. To compute  $EEF_{\text{sediments}}$ , ecological endpoints (e.g. LOECs and  $EC_{\chi}$ ) measured on a  $\text{weight}_{\text{MPs}}/\text{weight}_{\text{sediments}}$  basis were gathered from the literature and converted to chronic  $EC_{10\text{eq}}$ . The  $EEF$  developed is based on an  $HC20_{EC_{10\text{eq}}}$ , following updated recommendations. To aggregate the  $CF_{\text{sediments}}$  and  $CF_{\text{water}}$  obtained into *marine* CFs representing all marine species, two weighted aggregation approaches were proposed: 1-feeding behaviour weighting and 2-marine zones (i.e. pelagos, epi- and endo-benthos) species richness weighting.

High density polymers were found to have a larger residence time in sediments than in water due to their high sedimentation rate while low and medium density polymers have a longer residence time in water. The combined marine and freshwater  $EEF_{\text{sediments}}$  was calculated using data from 17 species, belonging to 3 taxonomic groups. The  $EEF_{\text{sediments}}$  obtained is 17.7  $\text{PAF}\cdot\text{m}^3/\text{kg}$ , significantly smaller than the  $EEF_{\text{water}}$  of 1067.5  $\text{PAF}\cdot\text{m}^3/\text{kg}$ . Obtained marine CFs range from  $1.83 \times 10^3$  to  $7.81 \times 10^6$   $\text{PAF}\cdot\text{m}^3\cdot\text{day}/\text{kg}$ , representing the affected fraction of total marine species. Overall, sediments are a sink for high density MPs and is the location where most of their impact occurs. It is therefore an important sub-compartment to consider in LCAs of products emitting high density MPs. However, low and medium density polymers do not impact sediments as significantly but have larger marine CFs, as their fate is longer in the water column where organisms are more exposed to MPs.

#### **5.06.P-We442 Plastic Litter in Life Cycle Assessment: Advances of the Marine Impacts in Life Cycle Assessment International Taskforce**

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Even though marine litter, especially plastic, is viewed as one of the main threats for biodiversity, life cycle assessment (LCA) was late to implement any indicators allowing the consideration of this important hazard. After the publication of a first framework detailing the assessment of marine plastic litter in life cycle impact assessment, the Marine Impacts in Life Cycle Assessment (MarILCA) working group has made concrete advancements in various stages of the impact modelling. This research proposes an overview of the projects that were led on the topic as a way of providing clear indications about the nature of the tasks that are either realized, upcoming or that constitute the next priorities.

For microplastics particles, the development of effect factors and fate factors have led to the publication of the first recommended characterization factors (CFs) for physical effects on biota, which lead to impacts on ecosystem quality. In parallel, various other research projects are focusing on deepening the fate processes, while other specific case studies are being led to foster the development of the methodology. Furthermore, the case of other substances contained within plastic products have also led to the development of additive-specific effect factors. Impacts of macroplastics are also being tackled and the modelling of impacts of plastic litter on other areas of protection, like human health and socio-economic assets is also underway, or partially completed. CFs are available to quantify the abiotic depletion impact of marine plastic litter.

The addition of plastic impacts in LCA is not without its challenges. Access to data is an important issue in the field, as data is developed simultaneously as the impact metrics are developed. Important tasks remain, namely the fate of additives, and the ability of plastic litter to act as vectors for other pollutants. Yet, the developed metrics have been tested in case studies and provided the first look at how impacts from plastic litter may compare to other impact categories considered in LCA. Results from these have also allowed the identification of important remaining challenges. The past years have led to significant steps in the quantification of plastic litter impacts, mainly in the marine environment and it is expected that the next 2 years should allow to the completion of the main impact pathways identified within the first framework.

### **5.06.P-We443 Updated and Comprehensive Characterization Factors for Microplastics in Life Cycle Assessment Considering Multimedia Fate Modelling**

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Plastic litter released into the environment poses potentially significant threats to ecosystem quality, human health, and socio-economic assets. Yet even today, Life Cycle Assessment (LCA) does not properly consider these impacts which can adversely affect decision making regarding plastic use. To address this issue, the international research group MarILCA (Marine Impacts in LCA) has proposed a framework to integrate the impacts of plastic litter in LCA. While MarILCA has primarily focused on assessing impacts in the marine environment, microplastics may impact other ecosystems depending on their emission locations. This project addresses this complexity by investigating microplastic transfer among other environmental compartments and establishing updated and more comprehensive characterization factors for a new impact category, *physical effects on biota*, using effect factors for aquatic, terrestrial, and sedimentary ecosystems.

To achieve this, a fate model is developed, building upon the SimpleBox4Plastics model, and adapted to the USEtox methodology. The model covers 9 environmental compartments (air, natural soil, agricultural soil, lake water, freshwater, marine water, and their sediments) on continental and global scales. It illustrates the fate mechanisms of microplastics within and between the compartments from their release to their complete removal.

From this model, fate factors are computed, quantifying the distribution of plastic emissions in the different compartments. Subsequently, midpoint and endpoint characterization factors are established for 9 spherical microplastics types: EPS, HDPE, LDPE, PET, PLA, PP, PVC and TRWP, each associated with distinct sizes (1, 10, 100, 1000, 5000 µm) using effect factors for aquatic, terrestrial, and sedimentary ecosystems.

This model allows for the first time the integration in LCA of potential impacts of nine microplastics in all compartments including sediments. The fate factors will facilitate a more precise characterization of microplastics' pathways in the environment. They could also be employed in the future to evaluate the impact of microplastics on human health. The characterization factors can be integrated with emission inventories, complementing life cycle assessments of plastic products and empowering governments and consumers to make more informed decisions regarding plastic use.

### **5.06.P-We444 Development of an Effect Factor for Quantifying the Physical Impacts of Macroplastic Ingestion in Marine Ecosystems**

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Plastic has become an essential material for satisfying numerous human needs. Due to its versatility and affordability, this fossil-based material has found its way into a wide range of applications and products. Despite the large benefits it provides to our societies, plastic products are often mismanaged. Since plastic is a very persistent material under most environmental conditions, with degradation rates reaching up to thousands of years, this mismanagement has led to its omnipresence in the environment, and especially in the marine compartment that serves as the ultimate sink for plastic waste. The unceasing influx coupled with the continuous circulation of oceanic gyres have resulted in the formation of marine debris hotspots in the open ocean. A recent estimate showed that between 1.1 and 4.9 million tons of plastics are currently afloat in the global oceans, a trend that increased rapidly since 2005.

The presence of plastic fragments in the marine environment is responsible for tremendous impacts on ecosystems. Marine species have been documented to ingest plastic debris, with records dating back to as early as the 1960s. Since then, around 1300 marine species from different trophic levels have been reported to ingest plastic debris, with trends showing a steady increase in consumption over time. The intake of considerable amounts of plastic debris by species can cause physical impacts (e.g., through damaging or blocking the gastrointestinal tract) which often lead to harmful and even mortal effects. Despite the increasingly good documentation of these impacts in marine ecosystems, current Life Cycle Assessment (LCA) methods still lack models for ingestion impacts of plastics. In this study, we present an Effect Factor (EF) for quantifying the physical impacts of macroplastic ingestion by marine fauna. We use previous ingestion records, species range maps, species functional traits, and marine surface plastic concentrations to derive a spatially explicit and species sensitive model. Our model paves the way for developing characterization factors that can be used in future environmental assessments of plastic products.

### **5.06.P-We445 Examining the environmental benefit of the chemical recycling of polypropylene plastic waste**

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The escalating global utilization and production of plastics since the 1950s has resulted in a significant and increasing carbon footprint. Plastic waste management, with 80% directed to landfills, 12% incinerated, and only 9% recycled globally, highlights environmental concerns. Traditional recycling methods, primarily mechanical, fall short of achieving a closed-loop process, necessitating the integration of the circular economy concept into advanced recycling technologies. Chemical recycling, particularly pyrolysis, emerges as a promising solution, allowing the conversion of plastic waste into original monomers for a closed-loop process. This study focuses on polypropylene (PP), a widely-used thermoplastic, and evaluates the

environmental advantages of chemical recycling, specifically pyrolysis. Using Life Cycle Assessment (LCA), we examine a scaled-up industrial process for the pyrolysis of sorted waste PP (wPP), considering two perspectives: waste treatment and propylene production. Energy integration, utilizing pinch methodology, addresses the process's energy intensity. The LCA, utilizing SimaPro with Ecoinvent v3.7.1 and ReCiPe Endpoint (H) V1.1 impact categories, assesses the cradle-to-grave scope. The first perspective analyzes pyrolysis as a waste treatment process, comparing it with incineration and landfilling, using 1 kg of wPP as the functional unit. Products obtained are considered avoided, and incineration heat serves as environmental credit. The second perspective evaluates pyrolysis as a propylene production process, comparing it with the business-as-usual (BAU) route. The functional unit is 1 kg of propylene, with credits for avoided wPP treatment. Results show positive attributes of pyrolysis in impact categories (human health, ecosystems, resources) due to environmental credits. Propylene and 1-butene exhibit the most positive influence. Incineration credits contribute to positive impacts. Landfilling poses threats to human health but minimal damage to ecosystems and resources. Comparing BAU propylene production with pyrolysis illustrates the latter's environmental advantages, even without considering credits. In conclusion, pyrolysis of waste PP proves a promising closed-loop alternative with environmental benefits. Further research into techno-economic aspects is warranted for widespread implementation.

#### **5.06.P-We446 The Importance of Material Flow Analysis for Life Cycle Assessment of Microplastics**

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Plastic pollution is a complex environmental problem that has gained interest from numerous fields of study. Given the persistence of plastic pollution in environmental compartments, impact assessment has become increasingly relevant. However, in order to carry out a comprehensive and holistic impact assessment using Life Cycle Impact Assessment (LCIA), the flows of plastic to and through environmental compartments must be understood. The challenge, however, is rooted in the inherent complexity in attempting to map plastic pollution sources and leakage pathways. It becomes increasingly clear that interdisciplinary work is required to accurately map plastic pollution. Therefore, we demonstrate the benefits of using material flow analysis (MFA) to populate the life cycle inventory (LCI) phase of LCA. By combining these methods, we were able to carry out an MFA of macro- and microplastic pollution from geotextiles, dollyropes and mulch film as well as a simplified LCIA of microplastics released from mulch film. The use of MFA allowed us to better understand macro- and microplastic leakage from relevant materials as well as highlight critical data and knowledge gaps that should be addressed in future research.

#### **5.06.P-We447 The Life Cycle Inventory of an Innovative Biorefinery for Polyhydroxyalkanoates Production**

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Petroleum-based plastics carry undoubted environmental burdens, thus, alternatives to fossil feedstock are sought. Biobased and biodegradable polymers, such as polyhydroxyalkanoates (PHAs), appear as a valuable substitute for conventional plastic. However, their environmental preferability over fossil plastics is greatly conditioned by the production process, whose high cost also hinders their market penetration. To be viable, solutions must consider both the cost and the environmental aspects. To overcome these challenges, an innovative PHA production process is proposed by the Horizon Europe-funded BioLaMer project, based on the valorization of food waste, through the food-eating black soldier fly larvae (BSFL, *Hermetia illucens*). The invariable chemical composition of the larvae constitutes a novel high-impact feedstock for the cost-effective production of PHAs and chitosan biopolymers. Life Cycle Assessment (LCA) is conducted from the very beginning of the proof of principle stage, to ensure the environmental sustainability of this innovative biorefinery and to provide a final product which is safe and sustainable by design. The project started in April 2023, and after the goal and scope definition, the Life Cycle Inventory (LCI) is now under development. Firstly, the main steps of PHA production will be here illustrated. The manufacturing process starts with the food waste to BSFL conversion, in a self-supporting larvae cultivation plant. Then, the BSFL exoskeleton is separated - for chitin extraction - from the protein and lipids fractions. These will be converted into PHAs through biorefinery. At the moment, various biopolymer synthesis pathways are trialed, comprehending both pure and mixed media cultures. As an accurate LCI is of primary importance to obtain reliable and strong conclusions, our first aim is to obtain a complete qualitative LCI comprising data on single-operation unit processes to the greatest extent, while minimizing black box unit processes. This not only allows easier reviews but also avoids multifunctionality problems as much as possible. Furthermore, while the quantitative data will vary as the production process is optimized, a detailed qualitative LCI is already relevant at this stage, to verify if available datasets exist for background processes. Moreover, it is also an essential base for the upscaling process, where lab-scale processes will have to be converted to pilot-scale plants.

#### **5.06.P-We448 Life Cycle Assessment of the Electron Beam-Assisted Production of Thermoplastic Elastomers Based on Recycled Polyethylene and Polypropylene Waste**

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Increasing production rates of rubber products combined with a lack of effective recycling methods have led to an exponential growth of rubber waste in landfills and incineration, inducing severe environmental problems. Due to the crosslinking of

rubbers, recycling via melt processing cannot be used to process emerging rubber waste. One way to recycle cross-linked rubber is the mechanical recycling into rubber particles (ground vulcanized tire rubber waste, GTR). This elastic filler can be used to produce thermoplastic elastomers (TPE). These composites are prepared by melt mixing of a thermoplastic polymer and GTR particles. The mechanical properties of GTR particle filled thermoplastics decrease proportionally to the percentage of recycled GTR particles due to low interfacial adhesion between the composite components. Recent studies have shown, that surface activation as well as the application of gamma ray-induced interfacial crosslinking did not enable the multiple recycling of GTR based TPE while improving its comparably low mechanical properties. Hence, innovative methods are crucially needed to enable and strengthen circular economy. A promising way is the application of electron beam treatment to produce recyclable TPE based on recycled polyethylene and/or polypropylene as well as GTR particles.

In this study, life cycle analysis was done to compare the environmental impacts of the cascade of mechanical recycling of rubber and mixed polyolefin waste on the one hand, and the conventional production of virgin rubber and polyolefin on the other hand. Under application of the LCA software Umberto, real industry data was combined with environmental database information. The results of this study will contribute to the understanding of current and future recycling processes and will help guide future research towards innovative solutions of the global plastic waste problem.

#### **5.06.P-We449 Life cycle assessment of advanced grade PLA product with novel end-of-life treatment through depolymerization**

*Spela Ferjan<sup>1</sup>, Anna Schwarz<sup>1</sup> and Josse Kunst<sup>2</sup>, (1)TNO, Netherlands, (2)Arapaha B.V, Netherlands*

Using biobased plastics has the potential to avoid fossil resource depletion and fossil CO<sub>2</sub> emissions. Polylactic acid (PLA) is a fast-growing bio-based plastic made from fermented sugars. Nowadays, PLA is used to replace fossil-based polymers in healthcare and single-use applications, such as for packaging applications. However, PLA offers a much broader application range with the targeted use of a combination of its stereoisomers; PL(L)A and PL(D)A. A variety of these advanced grades of PLA can be used for multiple purposes in durable consumer products such as furniture. Recycling complex, mixed material and advanced grades of PLA is currently limited, as mechanical recycling has limitations in recycling mixed PLA grades. Using a depolymerization technology, products of such advanced grades of PLA can be recycled to form high-quality recycled PLA. A cradle-to-grave life cycle assessment study was executed to evaluate the sustainability of high-end durable product (a rug) with mixtures of PLA grade and the novel depolymerization technology. The findings of the study showed a 70 % reduction in CO<sub>2</sub>-eq. emissions compared to a conventionally designed rug. However, an increase is indicated in the following environmental impact categories: land use, eutrophication, and environmental toxicity. Sensitivity analyses for collection rates showcased that design for collection and recycling are key to obtaining a more sustainable biobased products. Additionally, scenario analysis supported depolymerization for PLA as recycling technology with low CO<sub>2</sub>-eq. emissions. Based on the results of the LCA and additional scenario analysis, the use of PLA is encouraged to be used in more durable and lasting products, such as furniture, from an environmental perspective, provided that the products are designed for collection and high-quality recycling to ensure material circularity.

#### **5.06.P-We450 Updating Recycling and Substitution Ratios Throughout the Recycled Polyester Life Cycle**

*Heather Margaret Logan, PhD Candidate<sup>1</sup>, Valentina Rossi<sup>2</sup>, Juliane Hermary<sup>3</sup> and Anders Damgaard Pr<sup>4</sup>, (1)ENV, Technical University of Denmark (DTU), Kongens Lyngby, Denmark, (2)Sustain, Technical University of Denmark (DTU), Denmark, (3)Technical University of Denmark (DTU), Denmark, (4)DTU Sustain, Department of Environmental and Resource Engineering, Denmark*

As the EU transitions to a circular textile economy, it will be pivotal to use Life Cycle Assessment to assess which recycling routes provide the best trade-offs for recycling of post-consumer garments. As polyester makes up the majority of textiles in circulation today, it is imperative we explore the environmental impacts and benefits of polyester recycling throughout the value chain and consider how different blends, fibre qualities, and recovered quantities of post-consumer fibre play a role in viability of recycling routes, through the calculation of new recycling ratios. Moreover, due to the multiple products produced throughout the textile recycling and production process it is vital we consider how multi-functionality, and quality of recycling routes plays a role in calculating the substitution ratios for recycled fibres. Through combined Material Flow Analysis and Life Cycle Assessment scenario analysis of the post-consumer recycling value chain expected in Denmark in 2025 for polyester fibres we explore the role that the recycling ratios and substitution ratios play in creating robust and reliable assessments of polyester and polyester blended textile recycling futures.

#### **5.06.P-We451 Application of Life Cycle Assessment as a decision-making tool for eco-design in the development of an automated and digitalized production chain for speed-bumpers (AD CORSSI)**

*Pablo Francisco de la Reta<sup>1</sup>, Charles Göbbels<sup>2</sup> and Angélique Léonard<sup>3</sup>, (1)University of Liège, Belgium, (2)Reprocover, Belgium, (3)PEPs- Chemical Engineering Research Unit, University of Liège, Belgium*

Plastic recycling offers potential environmental advantages, such as reducing landfill and incineration rates and lowering greenhouse gas emissions and fossil resource depletion. However, a challenge is identifying applications where recycled plastic can effectively match the functional properties of the replaced virgin plastic, specifically the integration of recycled high-density polyethylene (HDPE) in the manufacturing of speed-bumpers for industrial vehicles.



The AD CORSSI project aims to develop an automatic manufacturing line to produce speed-bumpers made with recycled materials (recycled HDPE and recycled glass fibre). To achieve this, automation will focus on conveying recycled materials from the mixer to the mould, aiming to reduce variability in the current manufacturing process.

Currently, prioritizing environmentally conscious technological development is essential for a low environmental (including carbon) footprint and improved circularity. Eco-design at the early stage of research and development processes supported by Life Cycle Assessment (LCA), guides the development of zero-waste speed-bumpers and an automated manufacturing line. Additionally, it aids in comparing various end-of-life alternatives and contributes to developing optimization roadmaps for recyclability.

In this study, LCA quantitatively assesses the environmental impacts of producing a 25 kg speed-bumper with a 10-year lifespan and a resistance of 15 MPa. The LCA covers various HDPE waste feedstock, the production chain, usage and end-of-life considerations. Additionally, we conducted a comprehensive LCA comparison between the project-developed speed-bumper and conventional counterparts, examining three scenarios: polyurethane, concrete and the project-developed speed-bumper using recycled HDPE and recycled glass fibre.

Methodology Guidelines on LCA of recycled plastic products and Environmental Footprint 3.1 impact assessment method are used. The model is done in Simapro 9.5.0. A cradle-to-grave approach was followed, and the inventory was based on primary data collected from the plant and Ecoinvent 3.9 database was used as a secondary data source.

Eco-design strategies have led to a reduction in the environmental footprint of the speed-bumper developed in this project compared to current speed-bumpers. Recommendations for the most suitable materials and processes to facilitate the closed-loop recycling of speed-bumper will be provided.

#### **5.06.P-We452 Development of an Approach for the Comprehensive Life Cycle Assessment of an Epoxy Resin System in Relation to Toxicity Categories**

*Sabrina Sigourney Judie Diniz, German Aerospace Center (DLR), Germany*

Due to the large number of chemicals used in polymer systems, creating a complete Life Cycle Assessment (LCA) including manufacturing, use-phase as well as end-of-life, is a major challenge. This Topic is examined in more detail using the example of an epoxy resin system. In addition to the use of a resin, a large number of reactive diluents and hardeners are used, for example, to optimally adapt these systems to their intended applications. Due to this complexity, simplified reference systems have often been used in previous studies in order to keep the effort for the Life Cycle Inventory within manageable limits. To identify potentially important gaps in the data situation and other problems in the context of an Life Cycle Assessment of polymer systems, a reference system is compared with a product system supplemented with the help of a literature study with regard to the Life Cycle Inventory and the Life Cycle Impact Assessment. In order to be able to make a consistent comparison, the modelling is based on the methodology proposed in the literature, which also forms the background for the Ecoinvent database. To supplement missing data, a literature study is first carried out to determine the chemical composition of the reactive diluents and hardeners analyzed in more detail. The product system under investigation is then modelled using a commercial LCA database and modelling software. During this modelling, the diversity of possible manufacturing processes for individual essential base chemicals and the use of a supplemental database containing additional chemicals also play an important role. In order to be able to determine differences in the subsequent impact indicator results more precisely, special attention is paid to the toxicity categories in the context of the Environmental Footprint assessment method in addition to the frequently used impact category of climate change. To check for any deviations, a sensitivity analysis is carried out within these categories to recognize important differences in the impact indicator results in accordance with DIN EN ISO 14044. In this way, differences potentially influencing the impact indicator results of the aforementioned categories have been identified so far. Among other factors, they can be attributed to the use of different additives and manufacturing processes and should be given greater consideration in the future.

#### **5.06.P-We453 Lifecycle Challenges and Opportunities for Different Bio-Based Feedstocks**

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Climate-friendly and biodegradable alternatives to fossil polymers have long been sought as the world attempts to meet Net-Zero climate targets and curb plastic pollution. As such, biopolymers derived from biomass are often perceived as an ideal replacement for fossil polymers in various applications. However, the specific feedstock chosen to produce a bio-based material has a significant bearing on not just its physical properties but also its environmental impacts. Thus, it is crucial to distinguish between and consider the environmental benefits and drawbacks of biopolymers made of so-called first-generation feedstocks (i.e. edible crops), and second-generation feedstocks (i.e. inedible lignocellulosic biomass).

Here, we critically reviewed available studies containing production information on the biopolymers polylactic acid (PLA), lignin, cellulose, hemicellulose, cellulose nanocrystals (CNC), and cellulose nanofibrils/microfibrillated cellulose (CNF/MFC) and compiled a dataset on their consequent environmental impacts so as to visually map key environmental hotspots associated

with the use of different feedstocks. We found that edible, first-generation feedstocks used to create biopolymers like PLA create production hotspots due to impacts from fertilizer and pesticide use. Additionally, issues with indirect land use change and biodiversity loss were identified as concerns for these feedstocks. For second-generation feedstocks of agricultural residues/wastes used for hemicellulose, CNC, and CNF, production hotspots arise from the inefficiencies of both waste-based extraction processes and sourcing, if residues are considered "co-products", i.e. sharing the impacts of agricultural production, rather than burden-free wastes. Conversely, lignin, cellulose, hemicellulose, CNC, and CNF biopolymers derived from second-generation woody feedstocks are promising candidates for replacing fossil polymers, with low sourcing impacts due to the minimal chemical inputs required in forestry. Nevertheless, non-climate impacts such as biodiversity loss, land use change, demand for wood in other sectors, and the availability and upscalability of bio-based resources to substitute fossil polymers must also be considered. Overall, we present within this work a comparative picture of the lifecycle benefits and challenges associated with bio-based feedstocks that must be addressed prior to substitution of fossil polymers with biopolymers.

#### **5.06.P-We454 Life Cycle Assessment of single versus multiple use medical products: a case study for steam sterilization packaging**

*Tiffany Ramos<sup>1</sup>, Ofir Eriksen<sup>2</sup>, Ciprian Cimpan<sup>2</sup>, Thomas Christensen<sup>3</sup>, Asta Hooge Poulsen<sup>1</sup>, Kristian Syberg<sup>3</sup> and Benjamin Khoshnevisan<sup>2</sup>, (1)Science and Environment, Roskilde University (RUC), Denmark, (2)University of Southern Denmark, Denmark, (3)Roskilde University (RUC), Denmark*

In 2019, the European Union produced 53 million metric tons of plastic waste, of which plastic packaging alone accounts for a substantial 14 million metric tons. Projections indicate that this figure could surpass 100 million tons annually by 2060. This pervasive use of plastic products and packaging spans across all sectors, with healthcare being a particularly significant contributor. This surge in plastic consumption can be attributed, at least in part, to the widespread adoption of single-use plastic items and packaging, which have surpassed reusable materials, primarily for reasons of infection control, cost reduction, and convenience. Polypropylene (PP) specifically, stands out as one of the most common and prevalent polymer types, constituting a significant portion of medical waste. Products such as PP blue sheets or sterilization wraps represent a significant portion of waste, that are unpacked and discarded before a patient enters the OT, representing a potentially sortable non-hazardous fraction of waste at the time of disposal. An existing alternative to single-use sterilization sheets are rigid sterilization cases (RSCs), typically crafted from metal and designed for reuse. Both products (blue sheets and RSCs) are used to enclose wire baskets containing surgical tools for steam sterilization and were chosen for comparison to reduce excessive PP fractions of waste by reducing the use of single use sterilization sheets. Methodological tools such as life cycle assessments (LCA) have been widely acknowledged for systematically and quantitatively comparing the environmental impacts of two products or systems with the same function, such as the blue sheets and RSCs. This methodology serves as a valuable tool for comparing the environmental impacts of products (and services) throughout their lifecycles, encompassing everything from the extraction of raw materials to production, transport, use, and disposal (end-of-life management). LCA results measure the impacts caused by the products and services on human health, ecosystem functions, and the depletion of natural resources, in this case the CO<sub>2</sub> impact of both products were assessed. This study aims to compare the CO<sub>2</sub> impact of both products throughout their lifecycles to assess which would be the most sustainable product choice for healthcare facilities. We also address the implications of switching from blue sheets to RSCs to provide insight to these implications in practice.

#### **5.06.P-We455 Thermochemical decomposition and mechanical treatment of waste plastics as innovative and perspective technologies for production of recyclates**

*Tatiana Trečakova<sup>1</sup> and Vladimír Koč<sup>2</sup>, (1)University of Chemistry and Technology Prague (UTC), Czech Republic, (2)Department of Sustainability and Product Ecology, University of Chemistry and Technology Prague (UTC), Czech Republic*

The situation of waste plastics is problematic not only in the Czech Republic but also in the whole European Union. Despite the measures taken to minimise the use of plastics, the amount of plastic waste has been increasing in recent years. Neither the Czech Republic nor the EU do not have sufficient capacity to process waste plastics. The problem of recycling of waste plastics is exacerbated in the Czech Republic and the EU by the problematic approach to the application of produced recyclates on the markets. The production of recyclates from waste also touches on a very topical issue of decarbonisation, where the aim is to reduce dependence on fossil fuels in the economy. This can be partly solved by the production of recyclates from waste which would otherwise be unused as raw materials and would put a strain on the waste management system, hence the environment. Technologies of thermochemical decomposition and mechanical treatment of waste plastics has the potential to enable further development of the circular economy and to help solve the situation of self-sufficiency and raw material security. The construction and operation of technologies for the production of secondary raw materials/recyclates nowadays has also important role in terms of increasing raw material security. The issue of availability of secondary raw material is nowadays not only one of the most important environmental but also social issues. The possibility of replacing important primary raw materials with secondary raw materials from waste can partly contribute to solving this issue, of course with an informed and responsible assessment of the environmental impacts associated with the production of these secondary raw materials or recyclates. A life cycle assessment method (LCA) was applied to compare the environmental benefits of these technologies. The study shows that the technologies of thermochemical decomposition and mechanical treatment can be tools of reducing the impacts of climate change in waste management and promoting the material recovery of waste, which are also objectives of European Union's waste management policy.

#### **5.06.P-We456 Early-stage process design: Applying the Safe and Sustainable by Design framework to recycling of acrylonitrile butadiene styrene (ABS) plastic from electronics**

**Anna-Karin Hellström<sup>1</sup>, Nina Melander<sup>2</sup>, Anna Strid<sup>3</sup>, Diego Penalzoa<sup>3</sup>, Mattias Andersson<sup>3</sup> and Rolf Lindström<sup>3</sup>,**  
(1)Environmental Chemistry, RISE Research Institute of Sweden, Sweden, (2)RISE Research Institutes of Sweden, Sweden,  
(3)RISE Research Institute of Sweden, Sweden

In this study, we tackle the challenge of chemical contamination in the recycling process by focusing on the removal of brominated flame retardants from acrylonitrile butadiene styrene (ABS) plastic, a key obstacle in achieving a sustainable recycling within the circular economy.

Our approach, aligned with the Safe and Sustainable by Design (SSbD) framework, integrates circularity considerations from early design stage of recycled acrylonitrile butadiene styrene (ABS) plastic. We focus on the physical recycling process, where ABS is dissolved in a suitable solvent to separate and remove the flame retardants. This case study focused only on brominated flame retardants, although other additives with hazardous properties may also be present in the ABS.

Methodologically, we followed the SSbD framework's step 1, 2 and 4 beginning with a literature review and a non-target analysis to identify potential flame retardants in ABS. The RISE substitution tool, utilizing Hansen solubility parameters, guided our selection of appropriate solvents. These solvents underwent further process safety assessment, and in the final step, environmental performance was evaluated by incorporating input from lab-scale mass balance calculations using the WinGEMS process simulation tool.

A cradle-to-gate life cycle assessment was performed to identify hot-spots, which included a comparison with the production of virgin ABS from generic Ecoinvent data. Different scenarios featuring various mitigation measures were modelled, such as optimized filters, recirculation of solvents, treatment of direct emissions and substitution of solvents with alternatives from renewable sources.

The LCA results indicate that by implementing all of these measures, the chemical recycling of ABS process studied can achieve an environmental impact that is equivalent or lower than that of virgin production. However, while there is a significant reduction in climate impact, shifting to non-fossil-based solvents could create trade-off effects by increasing impacts in other categories such as water use, land use and eutrophication.

#### **5.06.P-We457 Decarbonization roadmap of the plastic industry in China through life cycle degradable plastics substitution**

*Fan Wu, Jinan University, China*

Plastics have become a crucial component of modern society, yet their enduring environmental impact poses significant challenges. In recent years, the spotlight has increasingly fallen on developing degradable alternatives to mitigate the long-term harmful effects of plastic waste accumulation. By integrating dynamic probabilistic material flow analysis (DPMFA) and life cycle assessment (LCA), we analyzed the dynamic changes in greenhouse gas (GHG) emissions during plastic life cycles, while clarifying the potential GHG reduction through degradable plastics substitution throughout the plastic value chain. Based on ARIMA, we estimated that from 2023 and 2030, China will produce 337 million metric tons (Mt) of PP, 263 Mt of PE, 239 Mt of PVC, 362 Mt of PS, and 554 Mt of PET. We constructed a DPMF network for these plastics, ranging from production to end-of-life management. The major applications for traditional plastics are packaging (38.68%) and medical textiles (31.91%), both of which have great potential for degradable plastic substitution. Through bottom-up analysis, we estimated that the cumulative substitution of degradable plastic in the next 8 years will reach 76.8 Mt of PLA, 0.995 Mt of PBS, 50.6 Mt of PBAT, 41.4 Mt of PHA, and 7.3 Mt of TPS. Furthermore, the GHG reduction potential and decarbonization roadmap for the plastic industry was estimated based on the substitution of each plastic during production and waste management considerations. The integrated DPMFA-LCA model can be utilized to forecast the future GHG reduction potential throughout the plastic value chain, while also providing reliable guidelines for decision-making in degradable plastic substitution scenarios.

#### **5.06.P-We458 Understanding Stakeholder Perspectives to Overcome Barriers in Tackling Textile Fibre Pollution**

*Miranda Prendergast-Miller, Abbie Rogers, Nkumbu Mutambo, Kelly J Sheridan and Alana James, Northumbria University, United Kingdom*

Microfibre pollution is ubiquitous across all environments (land, air and water) and is regarded as an emerging pollutant. While studies are underway to quantify fibres in different environments, we also need to understand the wider context of fibre pollution from the perspective of different stakeholders across the garment life cycle and stakeholder engagement with microfibre pollution as an environmental issue. A pilot study was conducted using semi-structured interviews with a range of stakeholders involved at various points in the fashion value chain, from sectors such as design, materials and manufacturing, and end-of-life (waste management). The interviews were conducted to identify the current understanding of microfibre pollution from different stakeholders and their perceived challenges in engaging in potential pollution mitigation strategies. Our findings revealed the breadth of agendas and actions across multiple actors and sectors and identified some of the drivers and barriers to change across the garment lifecycle. Our results converged around three key themes: awareness; governance; and stakeholder collaboration. The main findings are:

*Awareness:* microfibre pollution was recognised as an emerging pollutant, but there were significant gaps in knowledge, especially around impacts to soil environments; confusion in terminology; and a concern about the dominant focus on synthetic 'plastic' fibres, with little attention to 'natural' fibres.

*Governance:* there is a prevailing absence of data, baselines, thresholds and test methods, meaning that stakeholders currently have few or no legislative steers to act on.

*Collaboration:* there is a lack of industry response that engages stakeholders across the garment lifecycle.

Our findings highlight the need for multidisciplinary understanding of stakeholder perspectives to overcome the barriers to circularity in the fashion industry. This study reveals the need for data-driven environmental scientists to engage with stakeholders in garment design, materials and end-of-life to improve awareness of textile fibre pollution impacts, provide evidence to policy-makers, and to engage in cross-stakeholder collaborations. In this way, the environmental impact of fashion textiles can be fully considered within the garment lifecycle and help achieve sustainable circular practices for the industry.

#### **5.06.P-We459 Comparative LCA of two chocolate-bars packaging alternatives: the contribution of raw materials and End-of-Life**

*Francesco Arfelli<sup>1</sup>, Daniele Cespi<sup>2</sup>, Luca Ciacci Dr.<sup>3</sup>, Marlena Roguszevska<sup>4</sup>, Fabrizio Passarini Prof.<sup>2</sup>, Eleonora Rossi<sup>2</sup> and Gianluca Torta Dr.<sup>5</sup>, (1)University of Bologna, Italy, (2)Industrial Chemistry, University of Bologna, Italy, (3)Department of Industrial Chemistry "Toso Montanari", University of Bologna, Italy, (4)KB Folie Polska Sp. z o.o., Poland, (5)Department of Industrial Chemistry "Toso Montanari", University of Bologna, Italy*

Urbanization and globalization have fueled a significant increase in food and packaging production and consumption. Packaging constitutes approximately 2% of the gross national product in developed nations and plastics comprises the 40% of applications, raising concerns related greenhouse gas emissions and other environmental aspects. Therefore, there has been growing interest in exploring alternatives, with paper emerging as a prominent option. LCA has been widely applied to estimate the environmental impacts of both plastic and paper-based packaging, but comprehensive comparisons between the two alternatives are infrequent. This study aims to fill this gap by comparing the environmental performances of oriented polypropylene (OPPp) and paper-based (PBp) packaging of chocolate bars, encompassing industrial processing phase (IPP) and End-of-Life (EoL). The chosen functional unit is 1000 pieces of packed chocolate bars and the comparison is made on a cradle-to-grave basis. The OPPp and PBp product systems are modeled based on primary data from a Polish company and EoL scenarios are developed through laboratory tests. The study reveals that, excluding the EoL, PBp outperforms OPPp in 13/18 midpoint categories and the single score. Raw material contributions to Global Warming Potential (GWP) are approximately 34% for OPPp and 22% for PBp. Waste-to-energy (WtE) and recycling scenarios also favor PBp, with preferences in 10/18 and 16/18 midpoint categories, respectively. EoL contribution to direct GWP impacts is estimated at 43% for OPPp and 3% for PBp, with credits associated with avoided electricity production and avoided production of OPP and paper offsetting waste management burdens. In the recycling scenario, OPPp outperforms WtE for the single score and half of the examined categories, while for PBp, WtE performs better in only 3 categories. A sensitivity analysis suggests that increasing the renewable fraction in the energy mix could significantly reduce impacts. The study highlights the importance of accurate modeling of EoL using material-specific data to provide precise estimates. In conclusion, the study demonstrates that PBp packaging has lower environmental impacts compared to OPPp, but effective impact reduction requires addressing various life cycle phases, particularly the IPP. LCA proves to be a valuable tool for informing policymakers and increasing consumer awareness of the environmental impacts associated with packaging products and systems.

#### **5.06.P-We460 A Comprehensive Life Cycle Assessment of Aircraft Cabin Interiors from Cradle to Grave**

*Su Natasha Mohamad<sup>1</sup>, Rachael Rothman<sup>1</sup> and Anthony Ryan<sup>2</sup>, (1)Chemical and Biological Engineering, University of Sheffield, United Kingdom, (2)Chemistry, University of Sheffield, United Kingdom*

A wide range of plastic materials used in aircraft cabin interiors complicates segregation, and the lack of research in this field has demotivated recycling when the aircraft reaches the end of its life. This results in a heterogeneous mixture of plastic waste, leading to prevalent waste management practices such as landfill disposal and energy recovery. The intricate composition of these plastic mixtures poses significant hurdles for achieving a sustainable exit for these polymers and realizing a circular economy. Therefore, to address this issue, the study analysed the environmental impact of different waste scenarios for assorted plastic parts from the cabin interior of an aircraft, including the passenger safety unit, window panes, passenger seat, cabin wall, and many more, using the life cycle assessment (LCA) approach. Samples were retrieved from the Aircraft Interior Recycling Association (AIRA, UK), and secondary data were supported by literature. All measurements on samples were carried out with IRAffinity-1S Shimadzu Fourier-Transform Infrared Spectroscopy (FTIR). Among the identified plastics are fiber-reinforced plastics, polyetherimide, polycarbonate, nylon, polyvinylchloride, polymethyl-methacrylate, and polysulfone. To assess the environmental burden and sustainability performance of these plastics, a systematic evaluation of the environmental impacts associated with the production, use, and disposal of these interiors was conducted using SimaPro 9.5 software. The LCA followed ISO 14040 and 14044 standards, where the goal, scope, system boundary, functional unit, impact categories, and assessment method were defined. Besides that, a few waste scenarios were modelled to understand the trade-offs between landfill, incineration, and potential recycling of several types of plastics. Result shows that the cabin wall is responsible for more than 60% of total CO<sub>2</sub> emissions during cabin interior production, i.e., 110 tons CO<sub>2</sub>(eq.). Although the

weight percentage for polyetherimide (> 25%) is higher than fiber-reinforced plastics (~14%), the CO<sub>2</sub> emission for polyetherimide was only 24.4 tons CO<sub>2</sub>(eq.).

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#### **5.07 Prospective Life Cycle Assessment for Sustainable Solutions in Times of Environmental Crises**

##### **5.07.T-01 Prospective Life Cycle Assessment to Support the Assessment of Early Stage Bioeconomic Technologies in RDI Projects**

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Due to the climate and energy crisis the awareness for environmental assessments of products, technologies and sectors has increased and early stage assessments as well as future benchmark comparisons are required in many application fields. To support environmental assessments of early stage technologies, the life cycle assessment (LCA) community has developed prospective LCA frameworks. Ideally, pLCAs should accompany research, development and innovation (RDI) projects to support technology developer, but many barriers still occur to integrate pLCAs into RDI projects and accompanying RDI programs.

The purpose of this work is to make pLCA manageable in the context an accompanying RDI program. Therefore, we developed cooperation models and a stepwise procedure with an integrated upscaling step. For the upscaling step an existing pLCA framework was adapted to innovative bioeconomic technologies and the RDI program covering 10 RDI projects with approximately 50 project partners.

Based on the framework, the status quo and target scale were selected and the two upscaling mechanisms size scaling and technological learning were considered. Based on a classification scheme suitable upscaling modules for the pretreatment, conversion and downstream processes was selected in cooperation with the RDI projects and material and energy balances were compiled representing the current as well as the target scale of the investigated processes. These material and energy balances were used to construct LCI models. LCA results for the status quo and target scale assessment were calculated.

The LCA results show environmental impacts of selected RDI projects in different development stages and the contribution of scope 1, 2 and 3 impacts. RDI projects with low starting TRL show a high potential through upscaling, on average a reduction of 85%. For most of the technologies examined, the largest proportion of GHG emissions can be assigned to scope 2 emissions. This study demonstrates that further specifications of pLCA frameworks are necessary to make pLCA manageable in the context of RDI programs. This work suggest a stepwise procedure and cooperation models with data provider and adaptations of frameworks. Results indicate a significant reduction potential demonstrating the importance for pLCA for early stage technologies. The transparent implementation and interpretation help for non-LCA experts supports the consideration of LCA results in decision making processes.

##### **5.07.T-02 Life Cycle Inventory Estimation of Energy Use in Pharmaceutical Production: Upscaling from Laboratory data**

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In the last decade, the surging global pharmaceutical production has prompted environmental concerns, addressable through Life Cycle Assessment (LCA). However, LCA implementation is hindered by restricted Life Cycle Inventory (LCI) data, mainly due to intellectual property protections. Among LCI categories, energy inputs typically contribute over half of the impacts in chemical manufacturing. Estimating these inputs by Process Design Calculations (PDC) based on laboratory data emerge as the most feasible method. Despite existing studies often overlooking crucial environmental factors and limiting accuracy, our study aims to develop an improved estimation method for pharmaceutical processes, covering API synthesis and product formulation. The method incorporates all relevant factors for impacts such as global warming, using upscaling procedures and derived PDCs from laboratory data.

The developed estimation method followed a stepwise framework. In Step 1, existing upscaling procedures were adapted, integrating engineering principles and chemistry from an extensive literature review. Scale-dependent process parameters were determined from process engineering references and equipment manufacturers' catalogues. Step 2 incorporated average designs and equipment sizes to form a generalized industrial plant. Step 3 focused on modelling Process Design Conditions (PDCs) for each unit operation (UO), emphasizing equipment-specific factors such as heat losses. Different methodological approaches were applied based on context such as considering the presence of existing PDCs. The final step involved future validation through cross-referencing with industrial data.

The developed estimation method included 16 unit operations, 20 equipment types/designs, and 35 sizes, representing an improved and extended version compared to previous studies. The method employed PDCs to estimate energy usage for all defined UOs in pharmaceutical production, including precursor production. Various methodological approaches, such as parameter scaling, empirical data integration, and heat loss modelling were used to derive these PDCs under various contexts. This study is the first to develop an estimation method for industrial-scale complete pharmaceutical production using actual equipment design and performance data. The generalized method facilitated its application, improving efficiency and feasibility within an LCA context to supported all levels of LCA practitioners.

#### **5.07.T-03 Integrating Prospective Life Cycle Assessment in Energy System Optimization Modelling: Opportunities and Limitations**

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Efficient decision-making in emerging technology development is crucial in addressing global climate goals. This work explores the integration of Prospective Life Cycle Assessment (LCA) with Energy System Optimization Models (ESOMs) to enhance the evaluation of the life cycle environmental impacts of energy system transitions. While LCA assesses environmental impacts from a life cycle perspective, ESOMs guide cost-optimal pathways for meeting reduction targets, considering sector interactions. Existing studies coupling LCA and ESOMs overlook future energy mixes and life cycle inventories. This research bridges this gap by coupling prospective LCA with the EnergyScope TD model, focusing on the cost-optimal transition of the Belgian energy system. Using premise, a prospective background database is created supplemented by additional life cycle inventories for specific technologies based on primary data, manual upscaling, and process simulation. Environmental impacts of 111 energy technologies and 24 resources relevant for Belgium are evaluated using EF 3.0 and Cumulative Energy Demand (CED) to construct impact databases for EnergyScope TD. Preliminary findings on the climate change impact of a combined cycle gas turbine power plant show that prospective LCA mainly influences the construction phase rather than the operation phase. The ongoing integration enables an analysis of how these findings shape investments and operations across the energy system. The integrated approach offers opportunities to assess competition between technologies for renewable resources and identify optimal resource and technology utilization. Despite these advantages, limitations include the extensive manual alignment of assumptions between the premise database and ESOM. Additional challenges involve integrating the optimization-dependent end-of-life stage, limited consideration of impact categories beyond climate change in the optimization process, and ensuring coherent projections for various impact categories. In conclusion, this work provides a robust framework for coupling prospective LCA with ESOM, yielding a consistent database for technologies in whole-energy system transitions. Recognized opportunities and limitations set the stage for further analysis, including automating the coupling process, assessing the impact on the optimal transition pathway, and incorporating additional impact categories, offering insights for future energy system planning and policy-making.

#### **5.07.T-04 Dynamic-Prospective Life Cycle Assessment using Time-Explicit Life Cycle Inventory: Framework, Method and Case Study**

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Life Cycle Assessment (LCA) has become a widely accepted method for assessing the environmental impacts of products. However, one of its shortcomings is that it neglects the time-dependency of processes by assuming a time-agnostic Life Cycle Inventory (LCI) phase, in which all processes occur simultaneously and don't change over time. Such static LCA is ill-equipped to assess decarbonization pathways, for which both changes in production systems and the timing of emissions are

important. Two up-to-now largely independent fields of LCA, dynamic and prospective LCA, reform this time-agnosticism from different angles. Dynamic LCA (dLCA) captures the timing of processes and emissions in the LCI phase, allowing a time-dependent Life Cycle Impact Assessment (LCIA) step. Prospective LCA (pLCA) projects future changes in production systems, with recent advances in sector-wide inventory modifications resulting in modified background LCI databases for specific future years.

This work proposes a novel framework to link prospective LCI databases with dynamic LCA calculations. By combining the two LCA methodologies, we obtain a time-explicit assessment of the actual production systems using representative data for each process at its actual time of production and tracking the timing of processes and emissions. The proposed approach aims at automatically using the LCI from corresponding time-specific prospective LCI databases for each process with temporal information. The approach is based on the open-source python LCA framework Brightway25 and builds on the existing dynamic LCA package `bw_temporalis`. Initial results are presented for a suitable test case and compared with the results of a static, dynamic and prospective LCA, each applied individually. The challenges and limitations of this novel methodology are highlighted and easy-to-follow guidance for when this joint prospective-dynamic methodology may be a preferred option over established LCA methods is provided. The proposed framework can help to provide greater accuracy about the environmental impacts of long-lived products in a changing economy, highlight time-dependent trade-offs and guide policy makers towards more time-sensitive LCA-based decision making.

The focus of this submission is the description of the overall concept, its advantages, and initial case study results. A detailed description of the practical implementation of the method is given in a separate, related abstract (#22593).

#### **5.07.T-05 Strengths, Limitations, and Perspectives of coupling IAMs and LCA to study feasible and desirable societal pathways**

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Environmental transition planning requires to quantify the impact of activities in uncertain futures. Prospective life cycle assessment (pLCA) answers this need, lately building on integrated assessment models (IAM). Such pLCA practices are growing and needed but also raise new questions.

This study first aims to discuss the practical implications of IAM-LCA coupling to conduct pLCAs through an advanced experience of *premise*. Secondly, it proposes a step back on IAMs by reviewing critical literature and interviewing economists and IAMs specialists. Third, it points out promising avenues for pLCA future practices.

The practical challenges related to conducting pLCAs based on IAMs encompass: (1) no guideline to help selecting an IAM while different IAMs may lead to vastly different results, (2) the complexification of LCA results interpretation without specific guidance, (3) a limited interoperability of existing prospective databases, and (4) the change of a restricted number of sectorial technologies considered.

The main theoretical limitations reported in IAMs' literature are that, first, mainstream IAMs assume a continued economic growth, and rely on *dramatic technological progress* to make it compatible with Paris agreement' net-zero targets. Secondly, most mainstream IAMs do not consider the *energy requirement of the energy supply systems*. Decreasing energy return on investment of fossil fuels combined with energy required for renewable energy production could lead to an "energy trap". More generally, IAMs ignore the *biophysical embeddedness* of economic activities, e.g., energy and materials requirement: their mitigation scenarios may be unfeasible under biophysical bottlenecks. Finally, all IPCC's last report mitigation scenarios come from IAMs based on *neoclassical economics*, that strongly simplifies the economy and may lead to unrealistic projections.

To conclude, we should handle prospective LCA with caution. Aligning parameter values in neoclassical IAMs with historical data is a basic precautionary principle. We should consider further IAMs based on alternative economic schools of thought – e.g., post-Keynesian IAMs (e.g., E3ME) based on empirical equations, or building upon them, ecological macroeconomic IAMs (e.g., MEDEAS) considering biophysical embeddedness. Finally, we may want to untie from technocentrism and explore unusual socioeconomics, from simple down-scaling avenues (consumption decrease, low-techs) to a more radical degrowth.

#### **5.07.P Prospective Life Cycle Assessment for Sustainable Solutions in Times of Environmental Crises**

##### **5.07.P-Tu506 Future greenhouse emissions of all-solid-state batteries**

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All-solid-state batteries have emerged as an alternative to lithium-ion batteries (LIBs) in high performance electric vehicle application due to their promising performance in terms of energy density, safety and battery lifetime. This study evaluated the climate impacts of two all-solid-state battery pack, and compared to two lithium-ion battery packs under two scenarios with considering potential changes in background production processes between 2025-2050. To ensure a fair comparison, all battery

packs were designed for the same energy capacity, with the same pouch cell size. A battery-dimensioning model was developed to calculate the required amount of components for each battery pack, as well as the corresponding energy density. In this study, two functional unit were applied: 1kWh battery capacity and 1kWh of energy delivered over battery lifetime. The changes in background system were captured using the python package Premise.

#### 5.07.P-Tu507 Optimizing Energy Efficiency in Lab-Scale LFP Battery Cell: LCA Approach and Insights

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The increasing demand on battery energy storage technologies that are sustainable and efficient makes it important to assess a product's entire life cycle, from the extraction of raw materials to end-of-life treatment. In the context of LFP batteries, which are increasingly employed for their safety, longevity, and better environmental performance, a lab-scale LCA of a battery serves as a crucial tool for identifying and mitigating potential environmental hotspots stemming from its cradle-to-gate impacts.

In this particular field, there is a research gap, given the distinctive differences between laboratory-scale manufacturing and larger-scale production. These variations encompass unique energy consumption patterns, challenges in resource utilization, and technological limitations. This study aims to investigate the amount of energy, which is utilized by equipment in dry room operations, and during battery cell manufacturing.

A cradle-to-gate life cycle assessment is carried out for the battery cell. The functional unit considered is battery storage capacity measured in kilowatt-hours (kWh). Battery has a mass of 26g, average voltage 3.2V, and has an energy density of 92Wh/kg. Primary data is from the pilot line and respective background database processes is from Ecoinvent 3.9.1. Two scenarios were considered, where in real time scenario with 5 cells per day equal capacity 11.6. If the lab unit had a continuous scale of production, it could produce 16 cells per day with total capacity of 38.3Wh which would be the ideal case scenario.

For the manufacture of 5 LFP battery cells, the highest unit process energy consumption comes from the dry and formation room counting up to 86% of total consumption. Lab-scale operations may require more thorough testing, experimentation, and prototyping, potentially resulting in higher demands on the dry and formation room's-controlled environment. The Life cycle impact assessment showed that there is a reduction of GWP by 69% per cell manufactured if the laboratory unit was running in full capacity (ReCiPe 2016 midpoint-H).

The study makes particular recommendations for future research, such as investigating lab-scale energy dynamics in more detail, identifying potential hotspots along with a mitigation strategy, and also taking renewable energy integration into account. These intended suggestions seek to close the noted research gap and advance the continuous advancement of environmentally friendly manufacturing methods.

#### 5.07.P-Tu508 Potentials and hotspots of post-lithium-ion batteries: Environmental impacts and supply risks for sodium- and potassium-ion batteries

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Rechargeable batteries are one of the key technologies to reduce greenhouse gas emissions in the transport and energy sector. Nowadays, lithium-ion batteries (LIBs) represent the dominant market share of rechargeable batteries. However, concerns regarding the environmental impacts of manufacturing and requirements for critical resources result in the need for developing alternative battery technologies as well as improving LIBs. This study assesses environmental impacts and supply risks associated with the production (cradle to gate) of 1 kWh of two post-LIBs, including sodium-ion batteries (SIBs) and potassium-ion batteries (PIBs), against LIBs by using LCA and criticality assessment. Six types of current and emerging batteries are explored in this study:  $\text{Na}_{1.1}\text{Ni}_{0.3}\text{Mn}_{0.5}\text{Mg}_{0.05}\text{Ti}_{0.05}\text{O}_2$ -Hard carbon SIB (NMMT),  $\text{Na}_2\text{Fe}_2(\text{SO}_4)_3\text{-Na}_3\text{LiTi}_5\text{O}_{12}$  SIB (NTO), and  $\text{KFeSO}_4\text{F-Graphite}$  PIB (PIB),  $\text{LiNi}_{0.6}\text{Mn}_{0.2}\text{Co}_{0.2}\text{O}_2$ -Graphite LIB (NMC),  $\text{LiFePO}_4$ -Graphite LIB (LFP),  $\text{LiFePO}_4\text{-Li}_4\text{Ti}_5\text{O}_{12}$  LIB (LTO). The ecoinvent 3.7 and ReCiPe 2016 endpoint method (hierarchist) are used for background data and impact assessment method, respectively. The ESSENZ method is adopted to assess supply risks in terms of eleven categories. Post-LIBs showed comparable environmental performances and lower supply risks compared with LIBs. In particular, PIB showed the best performance for environmental impacts although being an immature technology compared to the others, which comprise a high degree of uncertainty. While LFP result was lower than SIBs (i.e., NMMT and NTO) in terms of environmental impacts, SIBs showed lower scores for supply risks. For the SIBs investigated, NMMT was better than NTO in both aspects. These results suggest that there is a trade-off among environmental, supply risk, and technological



maturity aspects. The environmental hotspots were NiSO<sub>4</sub> production for cathode for NMMT and NMC, and TiO<sub>2</sub> production for anode for LTO and NTO. KFSF anode and cathode had no significant environmental impacts, achieving the best performance. The LIBs show larger supply risks than the SIBs and PIB, attributable mainly to Li and Co used as electrode constituents. The results show the potential to develop sustainable battery systems based on SIB and PIB and support battery developers in identifying hotspots towards developing new-generation batteries with lower environmental impacts and supply risks.

#### **5.07.P-Tu509 Refining Life Cycle Assessments of Mobility Solutions: AI-Simulated Use Phase Calculations for Sustainable Transport**

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There are around 26 million electric cars on the road worldwide. Trends show that the market share of electric cars will be 20% in 2025. The use phase of electric vehicles is their main selling point, distinctively surpassing internal combustion engine counterparts in mitigating GreenHouse Gas emissions. However, the use phase of vehicles bases its results on rough estimates and assumptions, resulting in uncertain and unspecific data. One example is multiplying the average energy consumption (kWh/km) by the average lifetime kilometres travelled, resulting in the lifetime kWh per electric vehicle. These calculations for the use phase are very general and unspecific, especially when analyzing emerging technologies and low TRL vehicles. Therefore, we are currently working on improving the specificity and certainty of the use phase calculations of mobility solutions, such as people and goods transports, by simulating them in software which AI models support. This software can recreate a road network model provided with GIS data. This model is then populated with a fleet of vehicles, and the AI generates a simulated traffic flow. A validated model can be constructed by iteratively fitting these simulations to actual traffic counting. The validated model can then calculate the vehicles' consumption on microscopic and macroscopic scales, amongst other data outputs. A Frankfurt model has been created and is in the process of validation. This model will simulate a novel mobility solution by replacing the business-as-usual fleet with autonomous vehicles. This software is hypothesized to decrease the uncertainty of use phase calculations for upscaling scenarios of pilot lab phase vehicles. Therefore, it enriches prospective Life Cycle Assessments of emerging mobility projects by improving on a crucial aspect, the use phase.

#### **5.07.P-Tu510 Is it Really “Green”? Antisolvents for the Perovskite Solar Cell Production**

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The term “green” frequently appears in literature to describe materials with superior environmental footprint, driving researchers to prioritize these materials in technology development. The claim of “greenness” can be based on many factors, such as the material being biobased, biodegradable, or non-toxic, but unlikely to be supported by comprehensive life cycle data. In the case of chemicals, anisole, identified as a green solvent due to its low direct toxicity effects, has been explored for layer treatment in emerging perovskite solar cells technology. Solvent selection guides assess solvents based on criteria related to the environmental, health, and safety. For example, CHEM21 uses chemical properties such as boiling point and vapor pressure to determine the environmental and health risk of solvents during their use and end-of-life stages. Another guide, the GSK solvent guide, goes further to incorporate life cycle assessment (LCA) data in its scoring system. However, it should be noted that in some instances these inventory data are disclosed to be incomplete, yet the result of the assessment is presented as a single score, which does not fully convey the comprehensive environmental picture.

Conducting a full cradle-to-grave LCA on anisole and chlorobenzene used as an antisolvent in the production of perovskite solar cells revealed interesting results. Anisole demonstrated around 77-88% reduced ecotoxicity and carcinogenic human toxicity than chlorobenzene. Both had similar non-carcinogenic toxicity, with chlorobenzene edging out. In contrast, anisole's impact on climate change was significantly higher than chlorobenzene, emitting 6.20 compared to 2.83 kg CO<sub>2</sub> equivalent. Other categories, indirectly affecting human health, followed this trend.

This challenges the claim of anisole being "green," mainly attributed to its high production stage impact compared to the one-step synthesis of chlorobenzene. These findings stress the importance of conducting prospective LCAs early in technology development to ensure environmental sustainability.

#### **5.07.P-Tu511 Prospective LCA of Solid-State Fermentation Based Biostimulant Production: Unveiling Environmental Benefits**

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Innovative and science-based solutions are needed, especially in addressing global issues and environmental challenges. In this context, the role of Prospective Life Cycle Assessment (LCA) emerges as a vital tool. Prospective LCA envisions and assesses the environmental implications of emerging technologies, products, and processes. This is particularly relevant in sectors like agriculture, where valorization of residues and secondary raw materials for biobased products are essential in transitioning from linear to circular systems.

Biostimulants are widely used in the agricultural sector, aiming to enhance nutrient uptake and promote plant growth. However, the environmental implications of their production methods remain largely unexplored beyond the benefits they

offer. Generally, biostimulant production is energy and water-intensive and sometimes requires solvent extractions under stringent conditions. Thus, alternative biostimulant production approaches have been studied to reduce energy/water/chemical requirements by using low-cost and renewable sources, alternative processes, or green solvents. One of these novel approaches is the solid-state fermentation (SSF) of agro-industrial residues as raw materials, a promising method (batch scale - TRL 3) offering substrate versatility, low water and energy requirements, and limited waste generation.

The prospective LCA employed in this research covers SSF-based biostimulant production. Key parameters are systematically evaluated, including energy consumption, raw material extraction, transportation, and waste generation. The study aims to identify hotspots in the production process, providing insights into areas for improvement and sustainable practices.

The study further explores the feasibility of optimizing resource use, minimizing environmental impacts, and enhancing sustainability in low TRL bio-based technologies. This aligns with the overarching goal of prospective LCA—guiding ethical and sustainable innovation. This contributes to developing environmentally responsible practices in the agricultural sector and promotes the transition to circular systems.

#### **5.07.P-Tu512 Prospective Life Cycle Assessment of Flow Chemistry Production for Active Pharmaceutical Ingredients** *Kristie Tjokro, Valerio Barbarossa and Stefano Cucurachi, Leiden University, Netherlands*

To reach the objectives set within the European Green Deal, the pharmaceutical industry needs to decarbonize. Batch chemistry is the most common method for manufacturing active pharmaceutical ingredients (APIs). Continuous flow production has shown potential to be a greener, safer, and cheaper alternative to batch production, in line with the principles of the Safe and Sustainable by Design (SSbD) framework. Despite its advantages, flow chemistry for the pharmaceutical industry is still in its infancy. We conducted an ex-ante life cycle assessment (LCA) from cradle to grave to understand the differences between the two API manufacturing methods, thereby providing evidence of the environmental tradeoffs of transitioning from batch to flow chemistry. First-hand data is collected from pilot projects, thereby enabling the modelling of highly accurate production processes, while data gaps are bridged using stoichiometric approaches. Preliminary results based on a select number of material and energy flows show that implementing flow chemistry in place of batch production is associated with reductions in generated waste, greenhouse gas emissions, water use, primary energy use, and hazardous chemicals use. This work contributes to filling the existing knowledge gap on safer and more sustainable API production methods, therefore laying the groundwork for the operationalization of the SSbD framework in the pharmaceutical sector.

#### **5.07.P-Tu513 Safe and Sustainable by Design: Development of an Integrated Approach for Scale-Up Drug Discovery Using P-LCA**

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The pharmaceutical industry is one of the most important industries in the world. It is responsible for the research, development, and production of drugs, which are essential for human health and well-being. However, the production and use of medicines can also lead to environmental problems, such as pollution and depletion of natural resources. Additionally, there are risks associated with drugs that can have side effects on human health, both during treatment and indirectly in some parts of their life cycle. Safe and Sustainable by Design (SSbD) is a holistic approach that should help to address the sustainability aspects together with environmental and health risks associated with the production and use of chemicals and materials during the early stages of its development. For this, the general SSbD framework needs to be adapted to specific groups of chemicals/materials. It is therefore necessary to develop and operationalize e.g. a specific SSbD framework for the design of drugs to identify and mitigate risks and sustainability issues associated with the production and use of them and to support the assessment of new pharmaceuticals.

As a first step towards a “Drug-SSbD” operationalization, a procedure will be developed for the scale-up of inventory data needed for the full LCA of drugs, in particular nanomedicines. It requires the adaptation of an existing framework, originally designed for enhancing the environmental impact assessment of chemical processes, to align with the scale-up of industrial pharmaceutical processes. The synthesis of high-purity substances demands specialized raw materials and involves intricate production steps, introducing unique environmental considerations. The use of Prospective LCA (P-LCA), will help not only to evaluate and anticipate the environmental impact associated with production during the scale-up but will also improve the possibility of comparing lab-scale processes with industrial ones.

Simultaneously, the second research direction delves into exploring risk assessment (RA) tools during the early stages of development, using available data or in silico methods to predict the toxicity and ecotoxicity of a new drug. Customized Quantitative Structure-Activity Relationship (QSAR) studies can support the P-LCA, through methods like USE-tox, connecting RA and LCA.

#### **5.07.P-Tu514 Integrating Future Energy Scenarios into Prospective Life Cycle Assessment: A Case Study of Hydrogen Production Technologies**

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This study focuses on assessing the environmental impacts of hydrogen production through water electrolysis. The research explores two methodologies for integrating future energy mix scenarios into Prospective Life Cycle Assessment (pLCA): the direct adaptation of electricity backgrounds from established LCA databases such as ecoinvent and the integration of projections derived from Integrated Assessment Models (IAM) using the open-source Python library *premise*. The latter approach allows for comprehensive modeling of transformations in electricity and energy-intensive activities.

A case study was conducted on hydrogen production using Alkaline Electrolysis Cells (AEC) and Proton Exchange Membrane (PEM) technologies. Parameters such as electricity mix, electrolyzer efficiency, and stack lifetime are varied to model future scenarios based on prospective energy mixes from RTE's "Energy Pathways 2050." Three scenarios for the future French energy mix in 2050 are considered, with different proportions of renewable and nuclear energy.

The assessment of hydrogen production technologies involves 1MW AEC and PEM electrolyzers. The study evaluates stack production, balance of plant, iridium production (PEM), and electricity consumption in a cradle-to-gate assessment. The assessment tool used is *lca\_algebraic*, an open-source library based on the *Brightway2* framework.

The results of the hydrogen Prospective Life Cycle Assessment (pLCA) indicate low CO<sub>2</sub> emissions associated with current and future energy scenarios in France. A reduction in ionizing radiation is observed due to decreased nuclear power electricity. However, transitioning to renewable energy scenarios leads to shifts in eutrophication freshwater, materials resources, and land use impact categories.

An analysis of parameter contributions highlights that, even with a highly decarbonized electricity mix, electricity consumption remains a significant factor affecting results, while stack lifetime does not contribute significantly.

The study underscores the importance of pLCA in evaluating the environmental impact of future hydrogen production. Methodological comparisons reveal a trade-off between simplicity and accuracy. The case study emphasizes the environmental burden shifts associated with transitioning to renewable energy sources.

#### **5.07.P-Tu515 Using Prospective LCA for the Assessment of Circular Economy Measures**

**Anne-Marie Isbert, Sofia Haas and Anika Neitz-Regett, Forschungsstelle für Energiewirtschaft (FfE), Germany**

To reach the defined climate targets, it is essential to transform the sector with the highest share in greenhouse gas (GHG) emissions – the energy sector. This transformation comes with an expansion of newly built renewable energy plants, associated with increased material requirements and related GHG emissions. The circular economy (CE) approach, including measures for lifetime extension, resource recovery and circular design, is one way to mitigate these GHG emissions and to ensure a successful energy transition.

The study highlights the role of prospective Life Cycle Assessment (pLCA) as a method to assess the environmental impacts of CE measures. It shows, that the pLCA enables the assessment of CE approaches by integrating prospective aspects as changes in processes over time as well as time-shifting effects of CE measures into the traditional LCA model.

A pLCA is carried out for the CE measures lifetime extension and recycling for onshore wind turbines. Amongst others, the foreground model takes into account the electricity mix, production processes for materials, efficiency of the production processes, energy and material demand for reparation, refurbishment or remanufacture of the wind turbine as well as recycling processes. For all of these processes prospective data for material, energy and environmental flows are collected and the results of the pLCA are shown. An exemplary result for the required data, the pLCA of the future German electricity mix is shown, emphasizing the relevance of background adaptation, as the two calculations (with and without background adaptation) lead to significant differences in results for future years. By conducting a pLCA for the CE measures lifetime extension and recycling for onshore wind turbines, the study points out the necessary adaptation of foreground and background systems and the required data.

The presentation concludes by discussing how these results can be used for the identification of an optimal lifetime, the so-called ecological break-even. Furthermore, the question, how the presented pLCA methodology can be put into practice and to guide decision-makers toward implementing circular measures effectively for a sustainable energy transition, is critically discussed.

#### **5.07.P-Tu516 Prospective LCA of Carbon Capture for Utilisation Technologies: Key challenges in low-TRL Biorefineries**

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Carbon Capture for Utilisation (CCU) technologies could play an important role in climate change mitigation strategies. Nonetheless, most of the CCU technologies are not mature and, in most cases, they are emerging technologies at the lab or pilot scale. Despite the scale, there is a high interest in distinguishing which technologies have the most promising environmental outcome and discarding those whose potential environmental impact is higher than the benefits. For that purpose, prospective Life Cycle Assessment (p-LCA) has been pointed out as one of the most suitable tools. However, there are several limitations in the inventory quantification of Low Technology Readiness Level (TRL) technologies. Uncertainties about the processes scaling-up effect, process integration, and the up-scaling of lab protocols are a few numbers of examples of the issues that a p-LCA practitioner faces.

VIVALDI's project exemplifies this case. VIVALDI covers the capture of biogenic carbon from bio-based industries to the production of high-valued Organic Acids (OAs) such as 3-hydroxypropionic (3-HPA) as well as nutrient recovery technologies with TRLs 3 to 5. Most of the technologies are electrochemical or bio-electrochemical technologies with a limited number of scale-up or without any scale-up references.

The poster aims to provide an overview of the main challenges identified and the battery of strategies found in the literature to overcome this issue and ensure the right support for different decision-makers: researchers, industry, and policymakers. Among the strategies found, it should be highlighted the bottom-up approach modelling or filtering technologies based on the best-case assumption method. Moreover, it should be highlighted the relevance of the substitution approach and normalisation with fossil-based OAs counterparts as a solution for benchmarking and comparison. As a conclusion, the authors present a miscellaneous group of techniques used to complete the whole overview of a complete CO<sub>2</sub>-biorefinery.

### **5.07.P-Tu517 Decarbonizing Transportation: Exploring Chlor-Alkali Hydrogen's Potential**

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The quest for decarbonization of transportation has intensified in recent years, prompting research into alternative fuels that can mitigate environmental impacts. Among others, hydrogen is frequently mentioned as a promising energy carrier with a potential to significantly reduce emissions, especially for heavy-duty vehicles and public transportation buses traversing long distances without frequent refueling.

Chlor-alkali hydrogen, a by-product of the chlor-alkali electrolysis process, could be particularly advantageous as a transportation fuel due to its availability as a by-product of an established industrial process, thus minimizing the need for additional dedicated hydrogen production infrastructure. However, the environmental performance of chlor-alkali hydrogen compared to the conventional fuels has seldom been quantified, neither has the overall potential of its production yet been explored.

This study delves into the prospective life cycle assessment (LCA) of chlor-alkali hydrogen as a potential future fuel source for transportation systems. Focusing on the European context, this study evaluates the environmental impacts in comparison to conventional fuels and projects the extent of chlor-alkali hydrogen production and its potential to decarbonize transportation. The total distance achievable by buses or heavy-duty vehicles powered by this hydrogen is quantified, while the associated challenges and limitations, such as infrastructure, technical advancements, or scalability, are addressed. The results are interpreted with respect to temporal aspects such as the composition of the electricity mix or the evolution of hydrogen demand and its production.

Overall, the research aims to provide insights into the viability and potential contributions of chlor-alkali hydrogen to a cleaner transportation landscape. The findings of the study could inform policy-making, guide strategic investments, and drive advancements in sustainable transport solutions.

### **5.07.P-Tu518 Life Cycle Assessment and Sensitivity Analysis of Radioactive Waste from Decommissioning the Fessenheim Nuclear Power Plant: Case of the very low-level radioactive waste treatment and disposal center**

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The decommissioning of French nuclear power plants is expected to generate a large amounts of very low-level radioactive waste (VLLW). The current prohibition on reusing VLLW in France presents significant environmental challenges, as existing waste disposal centers face saturation by 2028, necessitating the urgent construction of new facilities.

While few life cycle assessments exist for nuclear power plant decommissioning, our study comprehensively focuses on managing VLLW. Centered on the 11,200 tons resulting from the Fessenheim Nuclear Power Plant decommissioning, our methodology emphasizes storage center construction, including packaging and transport processes. To enhance assessment reliability, we integrate a careful sensitivity analysis ( $\pm 30\%$  variation in each data element) and a Monte Carlo analysis within

the SimaPro software. Developed with SimaPro software and ecoinvent databases, this method enables a precise VLLW life cycle analysis, revealing pivotal findings influencing effective waste management strategies.

Our analysis highlights packaging and transport as a pivotal factor significantly impacting respiratory inorganics, releasing harmful substances that affect human respiratory health. Moreover, the transportation process contributes 3290 tons of CO<sub>2</sub> equivalent, significantly influencing global warming. The use of construction materials, particularly geomembrane HDPE and gravel, exerts notable impacts on various environmental categories, including human health, ecosystem quality, climate change, and resources. These materials significantly contribute to the overall environmental footprint during VLLW storage and management, emphasizing the urgent need to explore sustainable approaches to mitigate these impacts.

In addressing VLLW challenges, urgent innovative strategies are vital. Aligning VLLW treatment with international recycling norms, questioning its current radioactive waste classification in France, and exploring recycling possibilities are crucial for mitigating environmental impact. Additionally, reassessing VLLW as conventional waste and adopting post-demolition material recycling show potential in diminishing long-term environmental effects in nuclear settings. Our study highlights the importance of adopting inventive approaches to sustainably manage VLLW, providing substantial environmental enhancement and safer practices in nuclear decommissioning.

#### **5.07.P-Tu519 Consequential Life Cycle Assessment of Mycoprotein as Meat Alternative in the EU Market**

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This study investigates the environmental consequences of supplying more mycoprotein, a meat alternative produced from fungi, into European markets using a consequential life cycle assessment approach. We first defined a cradle-to-gate system based on secondary data taken from available literature and database, and applied marginal data for electricity to the foreground systems. The initial system was eventually expanded to include other systems that are not physically related, but are expected to be affected through market forces. We assumed that additional supplies of mycoprotein in the EU market will substitute animal meat, the degree of which can vary greatly depending on the type of the product and/or market, and indirectly leads to the by-products substitution as well. To illustrate this, we first applied perfect-substitution assumption as the baseline scenario for each point of substitution being identified, i.e., pork, beef, chicken, manure, leather, milk, and oil. We subsequently applied different substitution rates, which are estimated based on the own- and cross-price elasticities of these commodities sourced from the literature, as part of the sensitivity analysis. The environmental impact categories considered include global warming, cumulative energy demand, land use, and water use, which are calculated using software-integrated methods. We expect that the net environmental consequences of supplying an additional one kg of mycoprotein will vary to some extent under the two different scenarios: (1) perfect-substitution assumption (2) empirically-based substitution. Furthermore, we believe that the perfect-substitution scenario will give minimum environmental consequences due to the profound benefits of avoided emissions from livestock system. In addition, it is more likely that the energy use and enteric fermentation would be central to the environmental trade-off as they are the major sources of climate-related emissions in mycoprotein and cattle systems, respectively. To sum up, we expect that mycoprotein as a meat alternative can contribute to food security with minimum adverse consequences, particularly because it requires much less land than comparable livestock farming.

keywords: consequential life cycle assessment, food system, livestock farming, mycoprotein, substitution

#### **5.07.P-Tu520 Life cycle assessment for farmed fish**

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Life cycle assessment (LCA) of fish aquaculture is actively conducted in foreign countries such as Europe, the United States, and China, especially focusing on species such as salmon and sea bass. In Japan, however, there is a dearth of publicly available research in this area. One explanation for this situation may be the relatively low importance of fisheries certification in Japan.

Against this background, this study assessed the environmental impacts of farmed fish in Japan. Specifically, we focused on Japanese red sea bream (*Todarodes pacificus*) farmed in Ehime Prefecture in southwestern Japan. Japanese sea bream is an important cultured fish in Japan and is popular as an auspicious fish.

The aquaculture facility under study has a unique approach: an automated feeding system. This system uses AI to monitor fish feeding behavior and adjust feed amounts appropriately. The benefits of this system include economic gains for the fishermen through efficient feeding and environmental gains through reduced waste and marine pollution. In addition, the system eliminates the need to send a boat out for each feeding, making operations more efficient.

In addition to the automated feeding system, this study incorporated input data such as seedlings, bait, equipment, and vessel fuel, as well as output data such as dead fish. Data on seedlings has not been addressed much in previous studies due to a lack of data, but we were able to obtain key data for this study and incorporated it into the evaluation. We also incorporated into our evaluation the fact that the life span of an aquaculture net is approximately 5 years, which corresponds to two cycles of a sea bream.

For validation and comparison of the results of this study, we plan to clarify Japan-specific characteristics and issues, while taking into account overseas studies. Furthermore, while the calculations focus primarily on greenhouse gas (GHG) emissions, we plan to expand the study to different impact areas, such as eutrophication and acidification

#### **5.07.P-Tu521 Life Cycle Assessment of LIFE Smart Agromobility project**

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The primary sector, particularly agriculture, plays a significant role in greenhouse gas emissions, with methane and ammonia being notable contributors. Acknowledging the pressing need to tackle these emissions, this study focuses on data from the LIFE Smart Agromobility project in Spain, funded by the European Commission under the LIFE 2019 program. The project aims to produce vehicular biomethane from pig manure, aligning with sustainability goals and reducing the environmental impact of the primary sector. In addition to reduce pollutant gas emissions, the initiative contributes to the reduction of fossil fuel usage and decreases energy dependence by locally producing renewable fuel. The dual objective of mitigating emissions and fostering energy independence underscores the project's broader environmental and strategic significance.

This research conducts a comprehensive examination of emissions, employing the Life Cycle Assessment (LCA) as its predominant analytical framework. The principal goal is to carry out an analysis of environmental impacts and determine the most sustainable approach within the unique context of LIFE Smart Agromobility. Furthermore, this work incorporates Monte Carlo Analysis allowing for the exploration of potential variations in emissions. By simulating diverse scenarios based on varying manure compositions, the analysis becomes more robust, accounting for uncertainties and providing a more realistic depiction of the potential environmental impact. This probabilistic modelling acknowledges the inherent variability in real-world conditions, enhancing the study's applicability and reliability.

The significance of this research extends beyond a quantitative evaluation; it contributes to a qualitative understanding of emissions management complexities. The results offer crucial insights into the dynamic nature of environmental impacts, emphasizing the need for adaptive strategies. In a world marked by changing climates and evolving agricultural practices, the adaptability of emission management strategies is a mandatory. The study's outcomes could be used to inform policy decisions, guide sustainable practices, and inspire further research of environmentally conscious approaches.

#### **5.07.P-Tu522 Wood Buildings or Decarbonized Concrete ? A Prospective Life Cycle Assessment Perspective Coupling Forest Sector Modelling and Prospective Energy Scenarios**

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Wood construction is an important levee towards the decarbonization of the construction sector, replacing traditional materials such as concrete and steel. Its development is part of transition pathways towards carbon neutrality at national and European level, as well as roadmaps fostering a circular and sustainable bioeconomy.

The intricate involvement of wood construction in diverse supply chains, with multiple co-products and circularity loops underscores the interest of using Life Cycle Assessment (LCA) as a systematic and all-encompassing approach spanning the entire life cycle.

To conduct a thorough assessment at both product and sectoral levels, it is imperative to integrate future decarbonization and material recycling strategies for carbon-intensive industries like concrete and steel. This integration is achieved through the coupling of LCA with specialized tools.

This study explores prospective LCA (pLCA) based on the coupling of forest and wood sector modelling with future energy mixes scenarios integrating projections derived from Integrated Assessment Models (IAM). Based on the open-source Python library *premise* [1], the approach facilitates the comprehensive modelling of transformations in energy-intensive activities within the inventory along the upstream value chains.

Key variables were parameterized, such as penetration of low-carbon industrial processes, material recycling level, and advancements in transportation technologies.

#### **5.07.P-Tu523 Life Cycle Assessment of Magnetite Production Using Microfluidic Devices: Moving from the Laboratory to Industrial Scale**

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Magnetite nanoparticles (MNPs) have important applications in several industrial and scientific fields for the remediation of contaminated soil and water, for instance. Emerging technologies such as microfluidic techniques have been adapted to continuously synthesize MNPs showing appealing results, such as a narrower size distribution. Therefore, this approach might become important for producing MNPs to meet industrial requirements.

This work proposes a possible scaling-up of the synthesis of MNPs using many microfluidic devices working in parallel. Such an increase is envisaged to go from a laboratory scale to an industrial scale. In this context, our work aims at investigating what would be the environmental impacts of such an increase in scale and understanding the possible shifting of burdens among environmental impact categories and from one part of the product life cycle to another. Therefore, we carried out a life cycle assessment (LCA) study considering all of the steps related to the microfabrication of the devices and to the production of MNPs at both the laboratory and industrial scales, including materials, electricity, and wastewater generated.

The LCA results showed that the rivets suitable for device inlets and outlets and the chemicals required for the synthesis process have the highest contribution to all impact categories, i.e., 80 and 90%, respectively. These results thus contribute to determining the overall environmental performance of each step during the synthesis of MNPs. The contribution analysis reveals that the manufacturing stage has a contribution of 97% at the lab scale, while the operation stage shows a contribution of 82% at the industrial scale. Finally, a sensitivity analysis is performed to identify the possible scenarios for replacing rivets required to manufacture microfluidic devices.

This work reports on the first LCA study of microfluidic devices, which we are aware of, that consider scaling-up calculations. Furthermore, the industrial production of the MNPs introduced here is an innovative process whose environmental impact, to our knowledge, had not been analyzed previously. Therefore, this study contributes to studying this process' overall feasibility by providing detailed information on the implementation of the LCA methodology to assess comprehensively emerging technologies such as microfluidics.

#### **5.07.P-Tu524 Prospective life cycle assessment on carbon capture and utilization technology to decarbonise the steel and fertilizer industry**

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Decarbonising the steel and fertilizer industries is essential to achieve the net-zero climate goals, as these industries account for 8% and 0.93% of the global greenhouse gas (GHG) emissions respectively. There are several strategies to decarbonise the steel industry, e.g. electrification or using carbon capture technologies. Similarly, the fertilizer industry can produce green ammonia and urea based on renewable green hydrogen from water electrolysis. Another approach is to combine waste streams from the steel industry as chemical feedstock for the fertilizer industry, and thus creating a more circular economy. This is investigated in the EU H2020 INITIATE project.

The INITIATE technology re-uses residual gasses rich in hydrogen, nitrogen and carbon as a resource for fertilizer production (i.e., ammonia and urea), by using sorption enhanced water-gas shift (SEWGS) technology. After fertilizer production, the remaining carbon dioxide is sequestered. In this way, the carbon footprint of both industries is expected to be lowered. This technology is still under development (i.e., pilot scale). For a fair comparison between the INITIATE symbiotic technology and conventional ammonia and urea production need to be compared at similar technology readiness levels (TRLs). Hence, a prospective life cycle assessment (pLCA) was performed to determine the environmental impact of applying this technology on industrial scale and identify improvement options. In the assessment, upscaling to industrial scale and potential scenarios future electricity supply were explored.

#### **5.07.P-Tu525 Regionalized environmental burden-shifting from strategic metals supply for the energy transition: a prospective life cycle assessment**

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The global shift towards renewable energies for achieving net-zero emissions relies heavily on strategic and critical metals (SCM). Essential components of solar panels, wind turbines, electric vehicles, and energy storage systems, SCMs raise concerns due to potential environmental risks throughout their life cycle. The objective of this study is to perform a prospective Life Cycle Assessment (pLCA) of the cumulative demand for SCM up to 2050, to identify the SCM and processes in the SCM value chains that have the greatest impact, as well as the impact categories that contribute most to the endpoint damages. Finally, by regionalizing the inventories, the study seeks to analyze North-South potential burden-shifting in the energy transition due to SCM supply.

First, the cumulative SCM demand until 2050 is estimated using an International Energy Agency (IEA) database for 3 IEA scenarios: Net Zero Emissions by 2050, Announced Pledges Scenario, and Stated Policies Scenario. An extensive literature review of existing pLCA studies is then conducted to benchmark calculation methods and areas for improvement. A preliminary static LCA of the cumulative demand for SCM is then performed using ecoinvent 3.8 and IMPACTWorld+. This preliminary study, together with the literature review, allows the selection of SCM to be refined in the model. Next, the python library *premise* is used to generate prospective ecoinvent databases for each scenario, and the following changing variables are modeled: primary production location, routes, ore grade declines, energy, water and chemical requirements, waste generation, and land use.

The metals with the highest cumulative demand were identified as aluminum and copper, followed by graphite and nickel. The preliminary static LCA revealed that copper and aluminum exhibit the most significant impacts on human health and ecosystem quality. Expected complementary results will include regionalization of mining and refining processes, as well as the impact of declining ore grades.

In conclusion, this study will provide insights into the environmental impacts of SCM supply, with aluminum and copper emerging as focal points. Recommendations will be provided to adequately support environmental SCM policies, to anticipate which countries could benefit from the energy transition and which countries would bear the costs, through the lens of North-South disparities already at stake in the consequences of climate change.

#### **5.07.P-Tu526 A review of the application of Life Cycle Assessment in hydrogen and alternative carbon source integration in Electric Arc Furnace based steel manufacturing**

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The iron and steel industry accounts for an approximate of 4% of anthropogenic CO<sub>2</sub> emissions in Europe, and 9% worldwide, due to the massive use of coal. According to the World Steel Association, 1.88 Billion tonnes (Bt) of crude steel were produced globally in 2020 emitting 2.21 Bt of CO<sub>2</sub>. Electric Arc Furnace (EAF) approach can be identified as a less carbon intensive approach to steel production and an approach to realize the recycling of scrap resources. The proportion of crude steel in EAF worldwide remains at an average of 28%, and with the strict requirement for energy conservation and environmental protection, EAF steel production will continue to rise.

Hydrogen exhibits great potential applications in the steel and iron industry due to its high calorific value, good thermal conductivity and high reaction rate, though producing the hydrogen that is required for the full decarbonisation of the steel industry demands an increase in electricity production of 20%, and enhanced access to locally available cheap renewable electricity. Steel production also requires alternative carbon sources to replace the existing coal and coke use which contributes to an estimate of 40-70% of the direct emissions. There are alternative carbon sources for the EAF that can either be used as charged or injected carbon. Charcoal, biomasses, rubber tires and polymers are the current known alternatives.

This work will focus on the environmental impacts of integrating alternative carbon sources to the EAF with and without hydrogen integration. The analysis will discuss the environmental impacts of these sources with an emphasis on biomass alternatives. Biomass can be identified as a preferred alternative in steel industry due to its low sulphur content, high ratio of carbon to ash and high specific surface that will potentially improve metal quality and furnace productivity.

We will initially identify the currently available hydrogen based steel production technologies with their alternative carbon-sources. The results will be presented based on the application of ex-ante LCA in each integrated steel manufacturing technology. This work will contribute to the ex-ante LCA modelling of hydrogen and biomass based steel production systems by consolidating the methodological challenges associated with drafting the lab scale or industrial scale systems and identifying feasible alternative scenarios that can be applied in a wider and a commercial context.

#### **5.07.P-Tu527 Beyond Lifecycle Assessment: cross-disciplinary methodologies to address challenges with environmental impact measures**

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There is widespread global concern at the scale of the environmental, economic, and social impacts caused by the unrestrained growth in global fashion and textile production and consumption. Awareness of the extant environmental crisis facing the planet has necessitated a targeted call to action by the industry itself in addition to a range of associated stakeholders. Consequently, a wide inventory of Environmental Impact Assessment (EIA) metrics, methods and tools are increasingly being devised and utilised across the industry. However, complex, and globalised supply chains make EIA challenging from a traceability perspective, with practices of out-sourcing to second and third tier factories being commonplace. Furthermore, the understanding of the extent to which fashion impacts the natural world remains limited due to unreliable collation methodologies, segregation of the value chain, and utilisation of intangible indices. Unregulated self-reporting across the



industry has resulted in unverified and unpredictable information being publicly communicated, with much industry and policy action being based on this untrustworthy data.

Assembling critical knowledge from the scientific community, juxtaposed with design capabilities, this project questions the *reliability, authenticity and useability* of current environmental and social assessment methodologies and critically analyses established indices and certifications. Through the development of a secondary systematic literature review, holistic mapping of existing metrics, and engagement with a range of relevant stakeholders (designers, brands, manufacturers, retailers, textile recyclers, consumers etc.) via focus group activities and one-to-one consultation, this research proposes a methodological toolkit to aid future research spanning creative and scientific fields of knowledge. Furthermore, participatory workshops with the science and design communities have provided validation and further development opportunities.

Collaboration between creative and science disciplines is paramount in the critical understanding, contextualisation and provision of evidenced based data when measuring EIA across the fashion sector. The development of the toolkit enables knowledge transfer across disciplinary boundaries and has extensive impact beyond academia, embedding research into the development of solutions regarding the way sustainability is measured and perceived from multiple stakeholder perspectives.

### **5.07.P-Tu528 Dynamic-Prospective Life Cycle Assessment using Time-Explicit Life Cycle Inventory: Methodology and Implementation**

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Life Cycle Assessment (LCA) is a key tool for evaluating environmental impacts of products and services, but typically overlooks the time-specific nature of processes. Traditional LCA assumes a static Life Cycle Inventory (LCI) where processes occur simultaneously and don't change over time. Neglecting such temporal variations is particularly insufficient in the assessment of decarbonization pathways, where understanding both the transition of production systems and the timing of emissions is essential. To incorporate a temporal dimension in LCA, two fields of research have emerged. First, dynamic LCA (dLCA) tracks the timing of processes and corresponding emissions, e.g., scheduling an end-of-life process a certain time after a construction process. Second, prospective LCA (pLCA) takes projections for future production systems into account, e.g., incorporating rising shares of renewable electricity. However, a comprehensive methodology combining both approaches is lacking.

We present a novel framework for joint dynamic-prospective LCA that is embedded in the open source python LCA software *Brightway25*. The novel framework allows users to define temporal distributions for processes and emissions and automatically match them to corresponding time-specific prospective LCI databases. The resulting dynamic-prospective LCI accounts for both the state of technologies in the production system at the actual time of each process (from the prospective databases), as well as the emission profiles of the biosphere exchanges. To achieve this, we build upon functionalities of the existing dLCA package *bw\_temporalis*, using graph traversal and convolution to retrieve a timeline of processes with timestamps for each process of the production system. This timeline is used as a basis for modifications of the production system, introducing temporal copies of each time-distributed process. According to the timestamps, new links to corresponding processes in the prospective background databases are added, yielding an improved representation of the technosphere. In addition to these links within the production system, assigning temporal distributions to emissions allows for dynamic impact assessment.

The focus of this submission is the underlying methodology of the framework and its practical implementation. A detailed description of the overall concept and advantages of the framework as well as first case study results are submitted in a separate, related abstract (#23128).

### **5.07.P-Tu529 Handling Multifunctionality in Prospective Life Cycle Assessment – a Systematic Framework**

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Prospective life cycle assessment (LCA) supports environmentally guided decisions to be made in the early stages of the decision-making process. However, a key concern in prospective LCA research, such as in retrospective LCA, is the handling of multifunctional processes. In a retrospective LCA, the quality of by-products and their flow type (good or waste) is mostly determinable. Conversely, data of a prospective LCA is based on various explorative scenarios at different future points in time and fundamental assumptions about a future state. Likewise, the quality and flow type of the by-products may not be determined and might change over time, as the processing steps may differ between laboratory and commercial scale. Therefore, the characteristics of a process in terms of multifunctionality are variable in a prospective LCA in quality (e.g., price, substitution ratio) and flow type (good or waste). Hence, in a prospective LCA, handling multifunctionality entails additional pitfalls compared to retrospective LCAs. Despite existing frameworks focusing on handling multifunctional processes in LCA and frameworks for conducting prospective LCA, none adequately addresses the variability inherent to handling multifunctional processes in prospective assessments. From a systematic review of prospective LCA case studies and

the existing frameworks, we derived five types of variability in a prospective LCA that influence the handling of multifunctionality. The five types of variability identified in this work are (i) changes in product quality, (ii) changes in the technology setup, (iii) changes in the legislation and regulations, (iv) price changes and (v) changes in mass balance. Based on these parameters, a framework that guides practitioners to handle multifunctional processes in prospective LCAs systematically is developed.

#### **5.07.P-Tu530 Harnessing Machine Learning To Forecast Environmental Futures – Tomorrow’s Waterscapes**

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As the quest for sustainable and circular product and technology design intensifies, so does the need for early-stage informed decision-making. Ex-ante Life Cycle Assessment has been widely adopted to support such decision, but faces many challenges, for example the epistemic uncertainty of characterisation factors, used to study environmental and social impacts. One of the methods in literature to address this uncertainty is recalculating characterisation factors based on projected data from Integrated Assessment Models. However, the relatively complex and data-heavy Integrated Assessment Model calculations oftentimes do not match the scope of Life Cycle Assessment-studies.

In this study, we evaluate the potential of Machine Learning as viable alternative to Integrated Assessment Models for creating spatially explicit time-series of future characterisation factors. We demonstrate our approach based on the characterisation factors ‘AWARE’ and Water Scarcity Index, which assess the impact of human-induced water extractions in over 10,000 global watersheds. We developed multiple Machine Learning models trained on historical time-series from environmental datasets and corresponding characterisation factors and identified Deep Learning models like NBeats as well as gradient-boosting models like XGBoost, LightGBM to be best suited for the endeavour. Different temporal and spatial aggregations of the environmental variables were used to find the best suited input data for the machine learning models, taking computing time and accuracy into account.

Average surface temperature, precipitation, and consumption habits were identified as important covariates for predicting future water scarcity. Further feature engineering included clustering watersheds according to the scarcity behaviour over the course of the year. Cross-validation was used to control overfitting of the models. In certain regions, the Machine Learning models forecast decreasing water scarcity that diverges from the conclusions of other research in this area. This discrepancy underscores the need for additional validation and refinement of the models to ensure their accuracy and reliability in forecasting water scarcity trends.

## Track 6. Environmental Policy, Risk Management, and Science Communication

### 6.01 Bird and Mammal Risk Assessment: Implementation of New Approaches for the Study of Higher-Level Effects in Wildlife Toxicology

#### 6.01.T-01 Redefining the Terrestrial Vertebrates Risk Assessment: A Paradigm Shift Towards Next-Generation Approaches for Agrochemicals.

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The birds and mammals risk assessment (RA) approach has been mostly developed for pesticides, but in line with the One Substance One Assessment proposal should be extended to other areas. In addition, the reliance on animal-centric methods for pesticide safety assessment (SA) is facing growing resistance from society, pushing towards a paradigm shift in evaluating overall performance, sustainability, relevance to human health risk assessment, and ethical considerations. This emphasizes the need for a more flexible and purpose-driven approach in implementing New Approach Methodologies (NAMs) for SA.

With our work, we aim to generate a novel conceptual paradigm and the use of mechanistic information using NAMs and landscape frameworks for developing Next-Generation Environmental Risk Assessment (NGERA) to gather comprehensive risk information in a more holistic and efficient manner. This paradigm seeks to take a step forward for the assessment of Pesticides, Biocides and REACH substances focusing on integrated and more informative risk paradigms, based on simplified landscape models, population dynamics, and the variability of agroecosystems and agricultural management. This refined paradigm offers realistic outcomes and allows the integration of diverse regulatory initiatives, serving as a versatile and dynamic tool for comparing environmental effects, assessing aggregated exposure a combined impacts on target species, ultimately enhancing the reliability of risk assessments through optimized representation of real-world scenarios.

It aims to focus on the risk drivers triggering the impacts on biodiversity conservation and ecosystem services/functions. Integrating these models into the RA process for the different regulatory sectors, would provide a more comprehensive and realistic understanding of the environmental consequences, allowing for a more refined evaluation of potential harms and their consequences. This shift represents a holistic approach that considers not only the immediate risks posed by these substances but also their lasting effects on the environment.

To conclude, the proposed conceptual framework, integrating mechanistic information through NAMs and probabilistic NGERA, represents a purpose-driven effort to gather holistic risk information and reflects a commitment to advancing methodologies in response to evolving societal expectations through a paradigm shift.

#### 6.01.T-02 Think Twice Before You Chose Your Study Site – Identification of Focal Species Study Areas Under the Revised Birds and Mammals Guidance Document

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If the bird or mammal risk assessment of a plant protection product fails at the lower tier effect or exposure assessment approaches, refining the risk assessment by considering a specific focal species (FS) to replace the generic model species (GMS) is a crucial element. The FS is a real species that is present in the crop in focus or immediate off-field at the time of product application. Since the risk assessment for the FS needs to be sufficiently protective of all other species of the same feeding guild, the determination of the FS is crucial.

Therefore, the recently revised EFSA guidance document for the risk assessment for birds and mammals (2023) highlights the need to put more effort in the identification of the most vulnerable species per feeding guild as the most appropriate FS. It is now emphasised that not only prevalent and abundant species are to be considered, but also rare and/or more locally distributed species. Hence, already the selection of the study area to conduct a focal species study is crucial for not missing relevant species.

In order to consider all relevant species that might occur in the crop in focus or its immediate surroundings and at the respective time of year, we developed a stepwise approach to identify FS-candidates in winter cereals as an example.

In a first step, from a list of European bird species, all species that potentially use the crop are assigned to at least one feeding guild. For each of these species, their distribution during the relevant time of the year that is linked to the crop growth stage during product application is mapped. This step also takes into account seasonal distribution changes due to breeding, migration and overwintering. From this data set, regions in each regulatory zone are identified where preferably all relevant species (incl. rare and/or locally restricted species) occur and the crop is cultivated in a sufficient amount. We also give recommendations in case that no region can be found that covers all regionally distinct species.

A subsequent FS study following these theoretical considerations must ensure that all habitat types are covered and sufficiently replicated, in order to meet the requirements of all potentially relevant FS.

### **6.01.T-03 Seed treatment risk assessment scheme in the 2023 EFSA GD for birds and mammals: review and impact assessment**

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An impact assessment for seed treatment uses according to EFSA 2023 is presented using industry owned active substance data, and underlying risk assessment assumptions as well implications for the availability of seed applied technologies for farmers in context with the EU green deal are discussed.

For tier 1, the EFSA 2023 online calculation tool was used and failure rate was compared to EFSA 2009.

For higher tier, two approaches were used to refine the reproductive risk for the exposure to treated seeds scenario according to refinement options outlined in EFSA 2023: i) quantitative refinement by introducing focal species, PT, FTWA value in case TWA can be used (all higher tier parameters were theoretical to allow for better comparison between compounds); ii) qualitative refinement by calculating the maximum searching area to reach the RAD.

Our preliminary analysis suggests that the EFSA 2023 risk assessment scheme for seed treatment uses is not fit for purpose. At tier 1, failure rate for the reproductive risk assessment for the treated seeds scenario is >90% of the tested cases. The tier 1 risk assessment does thus not allow to effectively separate high from low concern products as is the case for spray uses. At higher tiers, high conservatism (e.g. assumption of *ad libitum* access to treated seeds) and limited higher tier options not realistically reflecting relevant field conditions (e.g. PT and PD cannot be used in combination) make it difficult to meet the relevant trigger for the majority of compounds. The resulting need for complex WoE evaluations is predicted to result in a high workload for regulators and in inconsistent evaluations for the same data set.

In the light of activities to reduce pesticide exposure in Europe, seed treatments present an effective technology to limit exposure to non-target organisms. However, the EFSA 2023 risk assessment scheme renders the availability of seed applied technology to farmers as highly uncertain.

We therefore promote an open discussion with relevant stakeholders (e.g. a workshop with regulators, EFSA, industry) with the aim to develop a timely update of the seed treatment risk assessment scheme that is fit for purpose and more realistic for field conditions.

### **6.01.T-04 Modulatory Effects of Aquatic Resources on Neonicotinoid-Induced Developmental Toxicity in Tree Swallows (*Tachycineta bicolor*)**

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Neonicotinoids are the largest group of insecticides sold around the world for decades. The widespread use of these insecticides in modern agriculture not only decreases non-target insect populations, which limits the availability of food for aerial insectivores, but also poses adverse effects on them due to acute and chronic toxicity. Agricultural intensification has also resulted in losses of wetland habitat and decreased availability of nutritious aquatic insect prey, which are enriched with polyunsaturated fatty acids (PUFAs), especially omega-3 fatty acids. Previous studies suggest wetland coverage and dietary PUFAs can improve growth of swallow nestlings. Thus, the use of neonicotinoid insecticides and loss of aquatic insect prey resources is suspected as major drivers for population declines in aerial insectivores worldwide. We hypothesized higher wetland density or oral supplementation of PUFAs may modulate the toxicity of neonicotinoid exposure in aerial insectivores. In 2022 and 2023, we conducted research on free-living tree swallow nestlings orally dosed with imidacloprid (0, 4, 6, 9, 13 µg/g bw) or clothianidin (0, 11, 23, 46, 75, 95 µg/g bw) in 2 experiments testing the additive effect of high or low wetland density or supplementation with high or low PUFAs (medaka fish oil or organic sunflower oil). Post-fledging survival and movement were also assessed by tracking the fate and natal site departure timing of nanotagged fledglings with the Motus network. Our results show that both insecticides significantly impaired the growth and condition of nestlings in a dose-dependent manner. Chicks reared in the high wetland density sites had higher blood omega-3 PUFA levels. In 2023, higher wetland availability boosted nestling growth and reduced imidacloprid-induced toxicity. However, short-term beneficial effects of co-exposure to PUFA-rich oil appeared limited. 22% of nanotagged chicks exposed to imidacloprid successfully fledged and were detected outside of their natal sites compared to 50% in control group and 60% in clothianidin exposed group. No significant differences were noticed for the time of departure from their natal sites. Together, these findings provide first evidence of acute neonicotinoid insecticide toxicity in swallows, and data in support for management and conservation of aquatic resources in agricultural landscapes to reduce risks of insecticide exposure and enhance growth and survival of aerial insectivores.

### **6.01.P Bird and Mammal Risk Assessment: Implementation of New Approaches for the Study of Higher-Level Effects in Wildlife Toxicology**

#### **6.01.P-We462 Validation of Avian In Ovo Assay for Sex Steroid Hormone Disrupting Properties**

*Andrea Rivero Arze, PhD, Elise Grignard, Pauline Lelandais and Philippe Hubert, Pepper, France*

Pepper (Public-private platform for the validation of endocrine disruptors characterization methods) is validating a method to determine the estrogenic and anti-estrogenic effects of pollutants in the embryos of White Leghorn chicken (*Gallus gallus domesticus*).

During the first phase of this validation (transferability phase), 3 laboratories tested 3 test items: 17 $\alpha$ -ethinylestradiol (EE2), fadrozole hydrochloride and genistein; with strong estrogenic, weak estrogenic and anti-estrogenic effects respectively. The objective is to test the method's repeatability and reproducibility as well as identify the improvements needed in the Standard Operating Procedure.

At day 4 of the eggs incubation, 20 $\mu$ L of the test item diluted in DMSO are injected to the air chamber. The experimental set up consists of 5 groups: a negative control of non-injected eggs, solvent control and 3 doses with 10x dilutions starting at the highest soluble non-toxic concentration. At day 14 the embryos are sacrificed and morphological endpoints as the size of the gonads and the length of Mullerian Ducts are assessed. Moreover, qPCR analysis of the aromatase gene expression is done taking samples from the embryo's left gonad.

Male embryos exposed to EE2 presented a significant increase of their left/right gonads surface ratio which represents a feminization effect. Females exposed to fadrozole hydrochloride presented a significant decrease for this endpoint. The Mullerian Ducts length measures allowed to determine an anti-estrogenic effect. Finally, the aromatase gene expression showed an increase for the males exposed to estrogenic compounds and a decrease for the females exposed to the anti-estrogenic substance.

The method was successfully transferred from the developer laboratory to 2 other naïve laboratories. The results showed a good intra-laboratory repeatability and inter-laboratory reproducibility. Some minor difficulties that were experienced during the transferability phase allowed to propose changes to the Standard Operating Procedure (i.e. taking out some irrelevant endpoints, modify DMSO volume, etc.). A new clarified version will be implemented for the next phase of the validation where the 3 laboratories will test 12 blind-coded test items.

#### **6.01.P-We463 Disentangling the Adverse Outcome Pathway of triazole fungicides on birds**

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Over the recent decades, a decline in farmland bird populations has been observed across Europe. This regression has been linked to agricultural intensification, a phenomenon characterized by different components, including the use of pesticides. Triazoles, a group of fungicides employed as seed coating treatment in crops, pose a potential threat to wildlife inhabiting agro-ecosystems, particularly granivorous birds that consume sown seeds. In Spain, these products are the most frequently detected in red-legged partridges (*Alectoris rufa*) hunted during the sowing season, with a prevalence of 29%. Triazoles can impact on the reproductive success of birds, reducing fertility rate and brood size. The aim of this study is to assess the reproductive effects on red-legged partridges exposed to seeds treated with tebuconazole and prothiconazole and begin to understand the mechanism by which triazoles may produce long-term effects on reproduction by carrying biometrical and histopathological analysis. Partridges were fed with wheat seeds treated at 0%, 20% or 100% of the labelled application rate. The exposure period lasted for 20 days in late autumn 2021, and reproductive parameters (including fertilization, hatching, and chick survival rates) were subsequently monitored in spring 2022 and spring 2023. In addition, gonadal size was assessed in 24 partridges euthanized at different experiment points, and tissue samples were collected for histopathological analysis. Significant reductions in fertilization, hatching, and chick survival rates were observed in couples in spring 2022, which fed triazole-treated seeds more than 4 months before. These findings agree with prior studies, that observed a 23% decrease in hatching rates and diminished brood size. No significant differences were found in the reproductive parameters checked in spring 2023. During histopathological analysis, different damages were identified, however no significant differences were found. The right testicle of birds that had consumed treated seeds was smaller than that of controls. The temporal delay in reproductive effects, occurring several months after triazole exposure in autumn, supports the hypothesis of disruption in steroid-regulated processes as the mechanism of toxicity. This study underscores the potential impact of use of triazole-treated seeds in agriculture on farmland bird populations and it is the first step to disentangling the adverse outcome pathway of triazole fungicides.

#### **6.01.P-We464 Predictive framework for estimating exposure to and risks of Second Generation Anticoagulant Rodenticides (SGARs) for kestrels (*Falco tinnunculus*)**

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Introduction: Transfer of an SGAR along the food chain is a relevant exposure pathway for secondary consumers, but the

European Chemicals Agency (ECHA) acknowledges that further research on non-target species, including raptors, as a basis for a quantitative assessment is required. In contrast, the European Food and Safety Authority (EFSA) revised guidance on risk assessment for birds and mammals outlines a tiered risk assessment scheme including exposure via secondary poisoning; however, it does not include rodenticides. Therefore, our study aims to combine the current ECHA guidelines for rodenticides with the new, improved EFSA guidelines, 2023, in order to create a predictive framework for better estimating the exposure of SGARs and risk in kestrels.

**Material and methods:** ECHA, 2018 secondary approach and EFSA, 2023 tier 1 and 2 were integrated into our modelling framework. The daily kestrel exposure (E) (ug/g body weight/d) to SGARs was calculated using the EFSA, 2023. E is compared with the toxicological benchmark of "No Acute Effect Threshold (NAET)" derived from endpoint median lethal dose (LD<sub>50</sub>). A value > 1 indicates that SGAR exposure represents a risk for the kestrel under the model assumptions.

**Results and Discussion:** Using field data of concentrations of four SGARs in rodents, we predicted that kestrels are daily exposed between 8.15 and 23.43 ng/g bw/d of brodifacoum, followed by bromadiolone (0.103-0.297 ng/g bw/d), difethialone (0.101- 0.292 ng/g bw/d) and difenacoum (0.046-0.135 ng/g bw/d). It is known that rodents have generated resistance to bromadiolone, so more toxic options such as brodifacoum are increasingly used. Moreover, brodifacoum has the highest log K<sub>ow</sub>, the longest half-life and the lowest liver elimination rate, indicating that brodifacoum bioaccumulates in rodents more than other SGARs. The risk characterisation ratios indicate that the calculated daily exposure to brodifacoum represents a risk (R) > 1. Although in the present study, difethialone has the lowest NAET (0.88), compared with brodifacoum (1.87), Difenacoum (166.67) and Bromadiolone (1788.27). Brodifacoum has a longer acetyl side-chain, which gives a high affinity to the active site contributing to its low LD<sub>50</sub>, making brodifacoum a highly toxic compound.

**Conclusions:** This predictive framework provides the opportunity to investigate the relative exposure and risk of SGARs to kestrels and other raptors under a range of ecologically relevant scenarios.

#### **6.01.P-We465 Higher tier refinement of the risk assessment for birds and mammals: Residue decline patterns in foliage food items**

*Kai Ristau, Christian Imholt and Roland Kramm, BASF SE, Germany*

Under the new EFSA Guidance Document (2023) herbivorous generic model species, in particular the small herbivorous- and medium herbivorous mammal feeding guilds, will show a high failure rate at the screening step/ TIER 1 for foliar spray risk assessments of birds and mammals as part of the authorization process for plant protection products in EU. In higher tier risk assessment substance-specific foliage residue dissipation data will likely be often proposed as a refinement to replace the default DT<sub>50</sub> of 10 d. In principle, when performing studies aimed at determining a substance-specific residue decline, the analysed food items should reflect the ones considered relevant for the risk assessment. For herbivorous mammals (or birds) the relevant food items are monocot and dicot weeds and/or crop plants for most crop systems (EFSA GD 2023, Annex A & B). There is, however, limited experience on how to execute field foliar residue studies with 'real' weed species as in the past it was a common approach to select crop species as surrogates for weeds assuming a similar dissipation pattern.

Compared to residue studies with crops field experiments with real weed species face several methodological challenges, e.g. the emergence behavior varies greatly among species and their composition is often strongly spatially - locally and across geographical regions - structured. In this project several field experiments in Germany and Spain were performed with a high variety of 'real' monocot and dicot weed but also crop- (wheat) plants. The field phase (foliar application + consecutive sampling of plant material) at all trial sites was completed in spring 2023 without perturbing influence by rainfall.

Different set-ups primarily aimed to realize a comparable temporal emergence of a sufficiently high number of typical arable monocot and dicot weed species (≥ 3, each), thereby reducing the temporal difference regarding timing of foliar application and consecutive sampling events for residue analytics.

Key lessons learned regarding the methodological challenges of field residue decline studies with real weeds will be shared in this presentation. Secondly, by assessing the obtained residue data the project aims to answer how the observed variation of residue dissipation can be attributed to the factors: 1) tested plant species (wheat, monocot and dicot weed species) and 2) geographical location – considering additionally the potential influence of dilution by growth.

#### **6.01.P-We466 Mixture toxicity assessments in birds and mammals according to GD 2023 – is the calculated risk real?**

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The new guidance document on "Risk assessment for Birds and Mammals" (BM GD 2023) provides an assessment scheme to address the risk from simultaneous exposure to multiple substances from plant protection products (PPP), referred to as "mixture toxicity". Different substances are assumed to act additive (cumulative) or over-additive (synergistic).

Although true synergistic interaction in mammals is a rather rare phenomenon, occurring at high concentrations and involves in most cases chemical groups that can be well defined (Cedergreen 2014), the new guidance (EFSA 2023) proposes a low threshold to assume over-additivity rather than additivity of toxicity. In case the calculated endpoint assuming dose addition is

by a factor of 3 higher than measured in the acute product toxicity study in mammals, EFSA concludes that a “mixture is proven more toxic than what is predicted the DA model” and consequently, “synergistic effects cannot be excluded”. To account for this uncertainty, an additional correction factor is calculated and applied to the risk assessment. Moreover, this correction factor is extrapolated to other exposure scenarios (from acute to long-term) and other taxa (from mammals to birds). In specific cases this approach might be justified. When applied in general it makes the risk assessment overly conservative, thereby resulting in a high and not justified risk for PPPs with several actives to dramatically fail the risk assessment.

We present a recent case of a mixture toxicity assessment, where the risk assessment in line with the new Guidance Document resulted in a complete failure of the acute risk assessment due to a calculated high product toxicity with presumed synergistic interactions of the involved active substances. The available dataset covers a range of acute studies on the active substances and a product study based on the Acute Toxic Class Method (OECD 423) with an LD<sub>50</sub> in between 300 and 2000 mg/kg bw/d, which does not allow to calculate a precise endpoint. Although, it was clearly demonstrated that one active is driving mixture toxicity, a high acute risk using the most conservative estimate for acute toxicity from the product study was identified. Finally, as limited refinement options are foreseen in the new guidance, further experimental data were necessary to prove the applicability of dose addition and avoid an overconservative assessment.

#### **6.01.P-We467 Refining Plant Residue Decline Data to Account For Decreasing Plant Water Content**

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For plant protection products, risk assessments for herbivorous mammals, such as voles and rabbits grazing in treated fields, are based on Residue Unit Dose (RUD) values which predict the concentration of an applied chemical on plant foliage in an animal’s diet, measured as mg a.s./kg plant per kg a.s./ha. Established RUD values are published in the EFSA Guidance Documents for the bird and mammal risk assessment, based on hundreds of residue trials where initial concentrations were determined after applications in the field and normalised to an application rate of 1 kg/ha.

RUDs for the category *monocot foliage* were established with residues from grass and, in large part, from cereal trials (share of 76%). Data from cereal trials was selected solely from early growth stages (BBCH 10-30), when plants are young, fresh, and tender, which represents the preferred diet of small and medium sized mammals.

Residue decline data in foliage can be used to refine long-term risk assessment, by calculating dissipation half-lives (DT50) to be used in time-weighted average exposure factors (fTWA). However, often the only available residue decline data is from applications in late growth stages (BBCH 60+), since these will result in the highest residues at harvest in the commodity assessed for human consumer safety. The number of samplings in these trials are often lower than recommended for wild mammal risk assessments. Thus, such trials at late growth stages are often not well suited for exposure assessments on herbivorous mammals which prefer young plant material in their diet.

We found that the residues in foliage often appear to increase in these late growth stages which seems to be the result of plant material drying out, leading to increased concentration (mg a.s./kg plant) in the remaining drier plant material. We suggest correcting residue data from late cereal growth stage trials for this effect to better match trial results with the paradigm behind herbivorous wild mammal risk assessment.

As part of the approach, we estimated the change of fresh plant mass over time for cereals. The results were used to adjust residues based on the reported plant growth stage at time of sampling. The approach was able to explain apparent increases of residue concentrations during trials and allowed us to derive residue DT50 for affected trials, for which kinetic evaluations were impossible before.

#### **6.01.P-We468 Time-to-Effect and Recovery of Effects in a Bird Reproduction Study Argues for Use of the Time-Weighted Average Factor (fTWA) for Eggshell Quality in the Regulatory Risk Assessment**

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Eggshell thinning is an undesirable adverse effect that is observed for some pesticides in bird reproduction studies and may lead to eggshell cracking and ultimately failure of embryonic development. The EFSA Guidance Document (GD) 2023 for bird and mammal risk assessment states that no time-weighted average factor (fTWA) shall be used for effects on eggshell thickness that is not correlated with maternal bw/bw change or systemic toxicity. This approach assumes that single-day exposure would be sufficient to induce the measured effect on eggshell thickness. In an avian reproduction with an insecticide (code BCS-CW64991), severe eggshell cracking occurred with increasing severity over time and an observable recovery of the response with a cessation of exposure. Specifically, dietary exposure of BCS-CW64991 to bobwhite quail (18 pairs per treatment group) was conducted for 21-weeks (10 weeks of egg laying) with half of the replicates terminated at that time and half the replicates carried out for a 4-week recovery period on untreated diet. The treatment groups consisted of 0 (control), 100, 300, and 900 ppm a.i. in the diet. Significant eggshell cracking was observed ca. 2-3 weeks after the start of egg laying. Eggshell cracking effects continued to increase over the duration of the laying period. At the end of the exposure period, there were significant effects on adult feed consumption and the number of eggs cracked among all treatment groups

(i.e., LOAEC  $\geq$  100 ppm). Additionally, the total number of eggs laid, the live 3-week embryos of viable embryos, the hatchability of 3-week embryos, the number of 14-day old survivors of normal hatchlings, and the body weights of hatchlings and 14-day old survivors were all significantly reduced at 900 ppm (i.e., LOAEC  $\geq$  300 ppm). Birds switched to untreated diet showed recovery with regard to eggshell cracking within 2-3 weeks. The presented case study demonstrates that the sensitive time window for exposure leading to effects on eggshell quality in bird reproduction studies may be much longer than the duration (approx. 1-day) of actual eggshell production. Thus, the current standard assumption in EFSA GD 2023 of short-term exposure leading to effects on eggshell thickness may well be overly conservative.

#### **6.01.P-We469 Applying a DEB-TKTD model to analyse avian reproduction study data: a case study with three fungicides and northern bobwhite**

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Sublethal effects to birds from exposure to plant protection products (PPP) are assessed through reproduction studies (OECD 206 and OCSPP 850.2300) in which several endpoints are measured (e.g., egg production, as well as mortality of adults and viability of offspring are monitored in addition to body weight and food intake of exposed adults). Through a specific experimental design, worst case exposure is assumed, and effect concentrations are derived. It is often observed that birds avoid feeding on treated feed, but this is typically not quantified or accounted for in the risk assessment. Assigning observed effects to food avoidance or direct PPP toxicity is possible with the application of mechanistic effect models. Here we applied the recently published avian DEB-TKTD model to analyse egg production data from studies with northern bobwhite. The model is based on a standard DEB model modified to account for the specific experimental design of reproduction studies and uses feeding observations as input. The new EFSA bird and mammal guidance recommends using TKTD models to provide a more realistic risk assessment, but this is challenged by the requirement for independent data for model validation which is at odds with efforts to reduce vertebrate testing. The model we applied here was partially validated with the reproduction study data and overall showed very good performance, therefore demonstrating its utility and ability to provide additional insights with the standard regulatory data. Based on observed feeding and concentrations in treatments, the model assigns changes in egg production to food avoidance, direct toxicity from PPPs in treated feed, or to a combination of the two. We present a case study with three fungicidal PPPs which show different combinations of effects from food avoidance vs direct toxicity. Fungicide A caused effects only in the highest treatment, of which 49% was due to direct toxicity and 51% was due to food avoidance. Exposure to fungicide B resulted in effects in the middle and highest treatment, where in the former 56% and in the latter 29% was due to food avoidance. Finally, exposure to fungicide C resulted in effects in all treatments and mostly related to direct toxicity: 76.5% in the lowest, 63% in the middle, and 96% in the highest treatment was due to direct toxic effects. We discuss these results in the context of the tiered approach and how it can inform risk refinement considering field relevance.

#### **6.01.P-We470 Benchmark Dose Modelling: Expectation vs Reality**

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In September 2022, EFSA (European Food Safety Authority) adopted a guidance document on the use of a benchmark dose (BMD) approach in risk assessment. The scientific committee (SC), who authored the guidance document, suggests that the BMD approach is more appropriate than a no-observed-adverse-effect-level (NOAEL), since in theory it makes more extended use of dose-response data and allows for quantification of the uncertainties in the data. In early 2023, EFSA released a revised bird and mammal guidance document (not yet noted) in which it states that a 10% effect level (EL10) should be used in the risk assessment rather than the NOAEL, and that this should be calculated using a Bayesian BMD tool created by EFSA.

A workshop was set up by EFSA to explain the background around the model and to provide clarity on use of the tool. The use of BMD was also covered during the 2023 bird and mammal guidance document workshop. Once the guidance document is noted, this analysis will need to be conducted on new and existing studies.

At CEA, we have run a substantial number of different datasets through the online tool and encountered numerous issues that we believe will become common due to the data generated in bird and mammal studies not being suitable for these types of analysis. The aim of this poster is to highlight the advantages of BMD, some of the challenges we have faced, and what we think needs to be improved for effective and meaningful use of the tool in the assessment of risk to wild birds and mammals.

#### **6.01.P-We471 Bird-Brained? Migratory Bird Risk Assessment According to the New EFSA (2023) Bird and Mammal Guidance**

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The new guidance document on Risk assessment for Birds and Mammals (EFSA 2023) contains several chapters dedicated to the choice of acute and chronic endpoints for use in ecotoxicology risk assessment. Chapter 5.2.9. "Special consideration for migratory birds" is a small section of the guidance, amounting to less than a single page, but with the potential to have significant implications for the avian risk assessment for some substances.



According to the guidance, the mode of action (MoA) of the substance must be considered to determine whether this could potentially lead to effects that could impact migratory birds e.g. ability to gain weight, changes in behaviour. The potential increased sensitivity of migratory birds is assessed by using the existing dataset, which may include passerine data (for non-EU submissions). The mammalian dataset can also be used to assess the risks to migratory birds if there is sufficient similarity between the effects observed in birds and mammals.

As this section of the guidance is brief, there are several areas of uncertainty in terms of which data are important for the assessment e.g. acute vs chronic, passerine vs other bird species, bird vs mammal data. It is not clear to what extent MoA should influence the risk assessment. It is also not particularly clear what criteria need to be met in order to discount the risk to migratory birds, nor when higher conservativeness should be triggered if concerns regarding neurotoxicity are raised. Finally, the presence of this section seems to have been prompted by concerns that migratory birds are particularly sensitive to neurotoxic insecticides, but no explanation or citations are provided to support this assertion.

CEA has now performed several migratory bird scoping assessments in line with chapter 5.2.9. The aim of this poster will be to discuss our experiences so far, in terms of the concerns raised in specific assessments and general issues they have highlighted.

#### **6.01.P-We473 Energy Content, Moisture Content and Energy Assimilation Efficiency by Birds and Mammals of Oil-containing Seeds and its Implications for Seed Treatment Risk Assessments**

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Energy content, moisture content and energy assimilation efficiency values are essential parameters in the food intake rate and thus exposure calculations for bird and mammal risk assessments. The recently updated European Food and Safety Authority guidance document on birds and mammals risk assessment summarizes these parameters for different food items relevant for birds and mammals. For seed treatments values for cereal seeds are usually proposed to be used for other crop seeds as well. However, oil-containing seeds (e.g. sunflower, oilseed rape) are expected to have a significant higher energy content compared to cereal seeds. This would result in lower food intake rates and thus exposure from consuming such seeds.

In order to be able to calculate reliable exposure values for risk assessment purposes, we conducted a systematic literature review to collect information on the energy content, moisture content and energy assimilation efficiency for oil-containing seeds (sunflower, oilseed rape, soybean, peanut, sesame, safflower, linseed (flax), white mustard and castor bean). The search yielded 361 papers, of which 111 contained values for at least one of the parameters of the crops in focus. The review of the bibliographic sections of these papers resulted in 40 additional publications with relevant values.

Only values obtained from raw seeds or kernel (peanuts and sunflower) were taken into consideration for calculations. Values have been calculated as the average of the means for each crop. Energy content value of oil-containing seeds were 24.25 KJ/g (N = 124, sd = 3.00), while that for moisture content was 6.62 % (N = 296, sd = 1.06). Energy assimilation values were only available for peanut, oilseed rape, soy, linseed and sunflower for a very limited number of bird and mammal species. Mean energy assimilation efficiency for mammals was 82.37 % (N = 13, sd = 5.00), while those for birds were 54.81 % (N = 14, sd = 9.61) for Galliformes and 79.51 % (N = 10, sd = 5.04) for Passeriformes. The values presented here can be used for future birds and mammals risk assessment, especially for seed treatment products used in the respective crops.

#### **6.01.P-We474 Outcome of a Virtual Workshop on Avian Higher Tier Studies Under the New EFSA Birds and Mammals Guidance Document**

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The new EFSA guidance document for the risk assessment for birds and mammals (2023) includes new requirements for higher tier studies but remains vague in the practical implementation of some aspects. In order to discuss practicable solutions to fill these gaps and find acceptable ways to conduct higher tier studies also in the future, a series of virtual workshops on higher tier refinement options for birds and mammals in the EU is planned for the following months.

The first workshop in this series will take place in March 2024 on avian focal species field studies. Stakeholders from industry, CROs, regulators and academia will be invited to join in an exchange at eye level. Practitioners in the field will present initial talks about the current status of higher tier refinement options and innovative approaches that would meet the new B&M guidance expectations. The talks will give an initiation for discussion of scientifically sound and yet practical ways of conducting and evaluating bird focal species field studies.

Participants have different backgrounds and opinions on the implementation of the guidance of higher tier studies as refinement options in the risk assessment of plant protection products. The workshop aims to find joint solutions to increase the trust in the study results for regulators on the one side and increase the security for industry that resource intensive field

studies are accepted by authorities when conducted appropriately on the other side. The results of this first workshop will be presented on this poster.

#### **6.01.P-We475 A Proposal on How to Consider ‘Vulnerable Species’ in Bird Focal Species Selection**

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The revised EFSA 2023 Guidance Document for Birds and Mammals emphasises vulnerability as criterion rather than prevalence for focal species (FS) selection. Weyers et al. (2022) suggest to rank FS candidates according to their expected magnitude of exposure by calculating a species-specific daily dietary dose (DDD). With this, species experiencing a high exposure would be ranked as potentially more vulnerable and are identified as candidates for focal species. The DDD is calculated using – among others – the estimated ‘proportion of diet an individual obtains from the (potentially) treated crop’ (PT). A real PT is assessed through a radio-tracking field study, but not for all species such field data are available. Here, the frequency of occurrence in the surveys (FO<sub>survey</sub>) conducted in each study field obtained from FS field studies is introduced as a proxy for PT in theoretical DDD calculations for the purpose of ranking FS according to their potential vulnerability. The presence of one or several individuals of a species during a high proportion of surveys – resulting in a high FO<sub>survey</sub> – could be an indication of a high utilisation and, thus, a potential high proportion of foraging time in this crop. In order to evaluate the suitability of this approach, the empirical PT values from radio tracking studies for different bird species were compared with the respective FO<sub>survey</sub> values for these species obtained in previously conducted FS studies in the same crop and BBCH stage. For this purpose, FS- and PT-field studies conducted for regulatory purposes were used as data source. Furthermore, the data was used to compare the ranking of FS candidates obtained using the new approach with the FS selection based on FO<sub>field</sub> according to the former guidance document. Based on a pool of ten case examples a positive correlation was shown between PT and FO<sub>survey</sub> as well as between FO<sub>field</sub> and the newly derived DDD, suggesting that the proposed approach may be suitable. Only in very few cases were additional species identified as potentially requiring further consideration compared to the former FO<sub>field</sub>-approach.

#### **6.01.P-We476 Health Status of Animals Tagged in PT Studies: the Importance for Regulatory Acceptance**

*Carmen Vives-Ferrández Sánchez, Benedikt Gießing and Olaf Fuelling, Tier3 Solutions GmbH, Germany*

In the EFSA Guidance Document on the risk assessment for birds and mammals, among the requirements and conditions for valid PT studies that should be considered, is mentioned that healthy individuals should be tagged. The Critical Appraisal Tool (CAT) for the evaluation of ecological field studies (Annex F of the Guidance Document) provides instructions for the reliability of a study in this regard, evaluating whether the general health and/or conditions of the animals studied are measured, described and reported. Thus, in order to fulfil this criterion, the field protocols and documentation of field studies must consider the well-being of the tagged individuals.

Several years ago, we started developing and using score sheets to describe the health status of the trapped individuals and further handling for animal welfare purposes. These score sheets have been constantly improved under consideration of comments from German animal welfare authorities. In our field studies, we use them to assess and document the general condition and the distress of captured individuals for both birds and mammals studies. The score sheets contain a list of possible situations and corresponding distress scores within three main sections: i) general condition of the trapped animal, ii) handling and measuring, iii) marking and tagging. It serves to evaluate the overall individual’s well-being that supports the selection of individuals suitable for tagging. Additionally, symptom-orientated aid measurements are given to support the recovery of the trapped individual if required. Clear criteria of symptoms are defined that prevents an individual from being tagged. Within our studies, the scores of each tagged individual are recorded during the trapping sessions. The animal welfare score sheets are filed as raw data and presented also in the reports. Moreover, in radio-tracking studies where the individual is continuously monitored for a complete activity period, its well-being is evaluated and recorded during each session in addition to the behaviour.

With such information on the health status available for the evaluator, this relevant criterion for the reliability of a study will be met. Therefore, we encourage using an animal welfare score sheets as a common methodology in the field studies and reflecting it in the reports.

#### **6.01.P-We477 GPS Tracking to Estimate Exposure of Birds and Mammals to Plant Protection Products for Risk Assessments**

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In the European registration process for plant protection products, a risk assessment must be conducted for birds and mammals following the guidance published by the European Food Safety Authority (EFSA). A realistic estimation of the exposure of a certain species to these products is a refinement factor used for these risk assessments. The exposure is defined as the proportion of diet an animal obtains from a treated area (PT) with the assumption that this is equal to the time an animal is active within this area over the course of a day.

Until now, conventional VHF radio-tracking data have been used in order to provide an estimate of the PT values. We want to demonstrate the use of GPS tags as a new method to collect data for PT estimations in accordance with the recommendations by the EFSA using GPS data collected during an experimental set-up as well as during field studies with European rabbits *Oryctolagus cuniculus* and field studies with three different bird species.

It will be explained how data can be rated regarding their accuracy in order to select only 'high quality data' for the PT estimations. Example data will be presented to show how 'inactive' tracking records can be identified, e.g. by measuring the level of the movement of the tag by an in-built accelerometer in combination with behavioural observations of the animals. Furthermore, we present an approach for estimating PT values using buffer areas around each recorded GPS location in order to account for positional uncertainty.

Both, GPS and VHF tracking are suitable methods to study home range utilisation of animals, but both have their advantages and limitations. An advantage of GPS tracking is the complete avoidance of disturbance by an observer and the possibility to continuously track animals with very big home ranges, thus of animals that would not be possible to be followed by an observer during a VHF tracking session. However, due to the battery size needed to collect positional fixes at short intervals for at least one day, GPS tags suitable for PT studies are only available for larger animals so far.

GPS tracking provides a useful tool for collecting monitoring data for regulatory risk assessments. We therefore recommend a careful consideration of the tracking method to use when collecting monitoring data for PT estimates as the more suitable method depends on the species to be studied as well as on other factors such as home range size and landscape characteristics.

#### **6.01.P-We478 Exclusion of Performance Outliers in Avian Reproduction Studies**

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Evaluating reproductive performance in avian studies using wild-type mallard (*Anas platyrhynchos*) and northern bobwhite (*Colinus virginianus*) can be complicated when birds perform less than predictably. A common practice in advanced statistical analyses has evolved in which the data from outliers is removed from comparisons. When poor performance correlates to bird injuries or poor health, removal of the data from statistical computations can reasonably be justified. However, when no apparent underlying reason for anomalies exist, is exclusion of outliers justifiable? Natural variation in reproductive performance occur in all life forms. Here we examine the occurrence of outliers in two key reproductive parameters: egg production and embryo viability to examine the frequency of occurrence of outliers and their correlation to biological causes. We use historical control data (HCD) from 40 mallard and 40 northern bobwhite reproduction studies, conducted at Eurofins over the past ten years, to determine the prevalence of outliers and their relationships to biological causes and ultimately on their influence in determining a statistical NOAEC. Our research provides a basis for discussions on differentiating types of outliers, their biological relevance and weight in statistical inference for avian reproduction studies.

#### **6.01.P-We479 Spatial Approaches to Field Study Characterisation and Representativeness to Address EFSA Guidance on the Risk Assessment for Birds and Mammals**

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New EFSA guidance on the Risk assessment for Birds and Mammals includes updates relating to the improvement of terrestrial vertebrate (TV) field studies for use in risk assessment of Plant Protection Products containing pesticide active substances. Within the guidance, two concepts are discussed: Field Study Characterisation and Field Study Representativeness. To address these concepts, a past terrestrial vertebrate field study was assessed and updated to address the new guidance needs for characterisation and representativeness. Field study characterisation involved collection and analysis of spatial data available for the study area and relevant to the species, including agricultural fields, grasslands, woody vegetation, forest, water/riparian areas. Percent area metrics were generated for several distances around the study fields, as well as examination of high-resolution imagery suitable for expert evaluation. Weather conditions during the field study were compared against 30-year monthly norms to verify the study year was not outside of expected variation from long-term averages. Field study representativeness was assessed at multiple scales, including EU, EFSA zone and Member State. The potential for species interaction between "habitat" (a proxy defined by spatial land cover data) with "use sites" (assumed treated fields by crop type derived from agricultural maps) was processed using gridded spatial data at a resolution of 10m. Shared border between habitat and use sites served as an interaction indicator summarized at the 1-km scale (i.e., how many of the 10m x 10m use site grid cells were adjacent to habitat cells). The 1-km scale is suitable for further aggregation at various sizes based on species attributes. In this case, local landscape interactions were assessed by developing "scenarios" a 5km x 5km summation of the interaction indicator which was quantified across all EU member states. The scenario(s) encompassing the study fields were placed into the full distribution of all possible scenarios (EU, EFSA zone, Member State) to assess the study location relative to all possible use site / habitat interactions (i.e., possible field study locations). The quantitative approaches developed here can also be used to prepare upcoming generic terrestrial vertebrate field study designs and support optimized study site selection to meet the new EFSA guidance requirements.

#### **6.01.P-We480 Long-term Toxicity to Birds and Mammals – Early Experience and Approaches to Benchmark Dose Calculation According to the Update to the EFSA Guidance Document**

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Under mandate from the European Commission, a revision to the EFSA Guidance Document for the Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7(12):1438) was published in 2023. It is anticipated that this Guidance Document will in the future become implemented for European regulatory assessment of Plant Protection Products. Amongst the updated content in this revised Guidance Document is a much-expanded evaluation of the appropriate toxicity endpoint for long-term risk assessment for birds and mammals. Central to this revised evaluation is the use of Benchmark Dose (BMD) modelling to define a specific effect level dose for relevant sub-lethal toxic effects to birds and mammals.

The aim of the work presented here is to develop pragmatic and scientifically justified methods to evaluate bird and mammal long-term data sets, to isolate and further analyse using BMD modelling critical long-term toxicity studies and affected parameters. Ultimately, the objective was to avoid unnecessary analysis for studies and parameters that would not yield reliable predictions, or where such a prediction would not inform the critical long-term endpoint suitable for use in bird and mammal regulatory risk assessment.

Using hypothetical datasets alongside the recommendations of the revised EFSA Guidance Document (2023) and established principles of ecotoxicologically-relevant effect determination, analysis and sorting of study effect parameters was undertaken, leading to an isolation of individual toxic responses which would potentially influence an overall critical long-term toxicity endpoint. These parameters were then evaluated for prediction of a defined effect level dose using the Bayesian Benchmark dose online tool provided by EFSA. Finally, predictions of a defined effect level dose for the study parameters analysed were evaluated for reliability, comparability to a corresponding No Observed Adverse Effect Level (NOAEL) and to ultimately conclude on the long-term toxicity endpoint to be defined for regulatory use.

The work undertaken shows the importance and time-effectiveness of prior detailed analysis of underlying long-term toxicity data sets, allowing for a focus on only those studies and parameters showing a dose-response, and which would potentially influence an overall long-term bird or mammal toxicity endpoint for regulatory risk assessment use. Furthermore, this up-front analysis can help to avoid generation of BMD predictions of poor reliability.

#### **6.01.P-We481 Storks and Pesticides in Rice Fields: A Feathered Perspective on Ecological Risk**

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Flooded rice fields form dynamic ecosystems, hosting many species crucial for ecological balance. Among these inhabitants, the white stork (*Ciconia ciconia*) stands out as a keystone species, playing an important role in the intricate web of interactions. This study concentrates on the central position of storks in flooded rice fields and their significance in assessing pesticide-related risks.

GPS tracking data from 220 monitored white storks and the publicly available database of bird counts, E-bird, particularly at rice fields were used to investigate the unique ecological dynamics shaped by storks in flooded rice field ecosystems. Storks of different ages were tagged in Portugal and tracked between 2016 and 2022. The GPS devices recorded individual's location every 20 minutes from the moment individuals were marked until GPS battery died or animals were no longer alive, as well as the bird behaviour (resting, flying or foraging). To provide a broader comparison for bird species in rice fields during the same period, the E-bird database (<https://ebird.org/portugal/home>) was accessed to obtain the details on sightings by experienced ornithologists in rice fields over the same years, highlighting the most commonly found bird species.

Storks were the species most commonly found in flooded rice fields during the annual periods between August and November. While in dry rice fields, storks were only present in much lower densities. During the rice flood season in Portugal, storks spend most of their time foraging on the rice paddies, feeding mainly on crayfish and small vertebrates. Therefore, it is clearly demonstrated from both sources of information that storks can be considered as a focal bird species for the risk assessment of plant protection products in flooded rice fields due to their abundance, time spent feeding on the field itself and the possibility of secondary poisoning by eating contaminated organisms.

In summary, this presentation underscores the unique role of storks in flooded rice fields and their potential importance as focal species in risk evaluation of pesticides used in rice. By honing in on storks as the primary species of interest, we aim to provide targeted insights that contribute to the effective management of pesticide use in rice.

#### **6.01.P-We482 Effects of Agrochemical Usage on Ecosystem Services Provided by Rice Fields**

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Rice fields not only are key to sustain human societies, they also serve as vital habitats for waterbirds. Therefore, it is urgent to assess the potential consequences of agrochemical for birds. Here, we employed the ecosystem services concept, suggested as a tool to evaluate the impact of chemicals at an ecologically significant level, to assess the influence of pesticide usage on two crucial services offered by rice fields: rice production and habitat provision for birds. We conducted surveys among farmers to gather information on pesticide usage, sowing methods, soil characteristics, rice varieties, field headland management, and yield. For the chosen fields, we characterized the surrounding landscape and field headlands and we performed bird point counts of 5 minutes of duration in 3 sampling periods during the rice growing season.

We collected information on 79 fields cultivated following the agri-environmental system and 22 cultivated organically (from now on conventional and organic). We assessed the importance of each variable for bird abundance and diversity and found that the size of the rice was the most important factor, with smaller fields presenting higher bird densities. Landscape characteristics were also important to explain the use of rice fields by birds. We found that bird community composition significantly differed between organic and conventional rice fields ( $p=0.009$ ). An indicator species analysis showed 6 species significantly associated with organic rice (all  $p\leq 0.04$ ), mostly related to headlands' vegetation. On the other hand, there were 5 species associated with conventional rice fields that breed or obtain their food from the in-field area of rice fields (all  $p\leq 0.04$ ). In this regard, it is important to mention that conventional fields are flooded approximately one month earlier than organic fields. In conventional fields, the abundance of most of these indicator species (5 out of 6) was negatively correlated with the usage of pesticides. Finally, we found that conventional fields were significantly more productive than organic ones ( $p<0.001$ ) but within organic ones, we did not find an association between pesticide usage and crop yield.

Our results can serve to guide management solutions to optimize the balance between the two main ecosystem services provided by rice field: productivity and biodiversity conservation.

#### **6.01.P-We483 Determining risks of triazole fungicides to birds and invertebrates under field conditions**

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The application of pesticides to crop fields supposes a release of deliberately toxic substances to the environment that is recognised as one of the main threatening factors to wildlife inhabiting agro-ecosystems. In this sense, triazoles are a group of fungicides widely used as foliar application and seed coating treatments to prevent from diverse fungal diseases. Triazoles act inhibiting the lanosterol-14 $\alpha$ -demethylase, an enzyme that is essential to produce ergosterol, a basic component of the fungal cell membrane. In animals, this enzyme catalyses the initial reaction of endogenous synthesis of cholesterol. Our previous research has shown that triazole fungicides may reduce cholesterol synthesis, decrease sexual hormone levels, and affect reproduction in birds, reducing fecundation rate and brood size. Besides these direct effects, triazole fungicides might also be affecting avian communities indirectly through the reduction of arthropod biomass that constitutes the basis for chick diet. Experimental studies show that triazoles can affect reproductive and developmental parameters in arthropods by disrupting the synthesis of ecdysteroids.

We aim to determine the risk of azole fungicides on wildlife associated with agricultural areas under field conditions. For two years we have been monitoring the abundance and diversity of birds and invertebrates in 30 agricultural locations in central Spain dominated by rainfed cereal fields and with different pesticide use. We performed four annual samplings at each location during which we censused birds and invertebrates, and collected samples of cereal sown seeds, soils and leaves of herbaceous and arboreal crops to determine the presence of pesticides (especially triazoles). We also recorded other crop and habitat management measures (e.g. presence of margins and fallow lands, fertilizer application). We present preliminary results on the diversity and abundance of fauna associated to cereal crops depending on the use of seeds treated with triazoles to test the hypotheses that (i) increased intensification will reduce abundance and diversity of avian and invertebrate communities, (ii) the use of fungicides, and especially triazoles, will play a significant role in that reduction given the reproductive effect caused by those substances, and (iii) some habitat management measure can modulate the effects of pesticides on farmland birds.

#### **6.01.P-We484 Impacts of triazole consumption on health and immunocompetence of passerine birds**

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The scarcity of natural food sources during autumn and winter forces granivorous birds inhabiting farmlands to consume sown cereal seeds. These are usually coated with triazoles, the most used fungicides in current agricultural practices. Recent studies with red-legged partridges have shown that their consumption affects the synthesis of sterols, which results in the alteration of endogenous cholesterol levels and steroid hormone regulation. However, there is a lack of information about the effects on passerine birds, despite their small body size makes the ingestion of a few coated seeds to result in high exposure doses. Additionally, despite the well-known interactions between steroid levels and immunocompetence, little is known about the impact of triazoles on the avian immune system. We developed an experiment in which infected and non-infected house

sparrows with avian malaria (a common avian blood parasite) will either consume triazole-coated or non-coated cereal seeds. We studied the effect of triazole treatment on body condition (calculated from length and weight), haematocrit (proportion of plasma and cellular fraction) and enzyme antioxidant activity in blood cells of birds. To estimate immunocompetence, we quantified white blood cells composition, avian malaria level of infection, and plasma levels of haptoglobin (a protein which increases with infection and/or inflammatory responses) and nitric oxide (an inflammatory and immune molecule produced to damage invading parasites). We hypothesized a decrease in the body condition, antioxidant enzyme activity and haematocrit, and an increase in the haptoglobin levels in infected and/or exposed birds. Moreover, we expected an increase in avian malaria parasitaemia and nitric oxide levels in triazole-exposed birds. These results could likely be used to provide a more integrated approach to the environmental risk assessment of these pesticides, in line with current efforts being taken at the EU level in this context, and to develop future conservation projects regarding farmland birds. Additionally, these results will contribute to the understanding of infectious disease dynamics and the evolution of host-parasite interactions under an anthropic-altered scenario.

#### **6.01.P-We485 Behavioural Responses of Imidacloprid-dosed Farmland Birds to a Simulated Predation Risk**

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Birds can use passive or active strategies in situations of risk when a terrestrial predator approaches and these behaviors could be altered by the ingestion of neurotoxic pesticides. Since behaviour is a sensitive indicator of the impact of an exposure and an early warning tool, the objective of this study was to evaluate the effect of sublethal doses of imidacloprid on the behavioural responses of the red-legged partridge to simulated predation risk. We evaluated the effect of 0 (C), 1 (T1) and 6 (T2) mg/kg bw exposure doses of imidacloprid on the behavioural responses of the red-legged partridge (*Alectoris rufa*) to predatory threat simulations. For this, 66 partridges, males and females in equal proportion, were challenged in groups or individually to intra and interspecific alarm calls, to a predator silhouette as an aerial predation risk simulation, or to a fox model as a terrestrial predation risk simulation. The antipredator behaviours were divided into active responses (escape, active vigilance) and passive responses (passive vigilance, crouching, freezing). Latency, percentage of individuals who responded, duration of each response, speed of active responses, and vocalizations, were also measured. Blood imidacloprid concentrations (mean  $\pm$  SE) were  $20.3 \pm 3.8$ , and  $153.8 \pm 22.0$  ng/ml in birds of T1 and T2 groups, respectively, and below detection in controls. Partridges of the treated groups showed a shorter duration of the crouching behavior against the visual predatory stimuli than controls in the group challenge experiment and a longer duration of the passive vigilance against the intraspecific auditory stimulus in the individual challenge experiment. T1 birds in the group experiment showed a trend towards a higher speed of escape compared to C birds. Conversely, T1 birds in the individual challenge experiment showed a trend towards a lower speed during the active vigilance compared to birds C birds. No effects of imidacloprid exposure were observed on other antipredator responses. The exposure to imidacloprid can influence the antipredator behaviours of red-legged partridges, compromising their capacity to freeze, hide and be unnoticed by predators. Subtle neurobehavioural changes caused by sublethal exposures should be considered in the environmental risk assessment of all the neuroactive chemicals because of the consequences on survival under field conditions where predation is a main driver of population dynamics.

#### **6.01.P-We486 Contamination of Second Generation Anticoagulant Rodenticides in the Eurasian Sparrowhawk and Assessment of Population Level Effects in the UK**

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We report levels of contamination of second generation anticoagulant rodenticides in the UK Eurasian sparrowhawk over a 30 year period. Using this data we predicted mortality rates induced by this exposure. Those mortality rates were applied to a population model to predict how sparrowhawk populations in four regions of Great Britain would have changed over the last three decades had the predicted SGAR-induced mortality not occurred. The population model combined British Trust for Ornithology on abundance of birds, mark-resighting of birds and nest records. The population model provides us with estimates for survival of different age classes of sparrowhawks (immatures, recruits and adults) and breeding success of birds of breeding age. We included effects of rainfall on immature survival, and density dependence effects of the number of breeding birds on breeding success. We then used this fitted model to ask how different sparrowhawk population trends may have looked over the previous 3 decades assuming different levels of mortality from SGARs. Using this approach, the model estimated the actual trend in sparrowhawk population over time and observed counts of birds. We assumed that the observed data and trend includes a hypothetical effect of SGARs. Therefore, we used the model to ask what the sparrowhawk population could have looked like, is that hypothesized level of SGAR induced mortality were not present in the population. Results are consistent with SGAR related mortality potentially being influential at the population level in some regions of Britain

#### **6.01.P-We487 Could the Use of Anticoagulant Rodenticides Against Plagues of Rabbits be the Cause of High Exposure in Predators and Scavengers?**

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Nowadays anticoagulant rodenticides (AR) are widely used throughout the world for the effective control of rodent pests. However, it is suspected that these biocides could also be used against non-target species, such as rabbits, whose significant damage has been reported in some regions of Spain. It is well known that predator and scavenger species may suffer secondary poisoning by ARs when they ingest contaminated carcasses.

Liver from dead animals is generally the most commonly used sample for biomonitoring short- and long-term exposures to ARs. However, blood has hardly been used for these purposes. Despite this, at least from a theoretical point of view, the blood sample can be really interesting to evaluate scenarios of recent exposure to these biocides.

The hypothesis of this study is that predators and scavengers would be more exposed to ARs in areas affected by rabbit infestations because of the illegal use of these biocides to control this plague.

This study aims to evaluate, by analyzing 78 blood samples, the degree of recent exposure to 5 first-generation and 5 second-generation ARs (FGAR and SGAR, respectively) in two populations of griffon vultures (*Gyps fulvus*) from the southeast of Spain. Vultures were captured and sampled at two vulture feeding stations, 200 km apart, during 2018 and 2019, one of them located in a region affected by rabbits and the other in a region free of rabbit damage.

The results indicate that 95% of the vultures sampled had measurable levels of AR in their blood. Forty-percent of the samples were positive to the FGAR, while in the SGAR the prevalence was 93%. The relatively high frequency of detection of FGAR was due to the presence of diphacinone in the samples collected in the region affected by rabbit damage.

On the other hand, in general, the high incidence of SGAR, especially difenacoum (92.5%) and brodifacoum (80%) in both locations, indicates that these animals have been exposed to a quantity of rodenticides above that expected in a recent period.

Anyway, it is evident that the griffon vulture, as a scavenger species, is exposed to rodenticides (some of them unauthorized), so we must assume that other scavenger species, as well as predatory species, must be suffering a similar exposure to these biocides, and particularly in the affected areas by rabbit damages.

Acknowledgements: Thanks to all the collaborators who participated in the sampling. In loving memory of S. Espín.

#### **6.01.P-We488 Evolution of Plumbism in Waterfowl From “El Hondo” Natural Park (Southeastern Spain) since 1998**

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El Hondo Natural Park is a wetland that was created in the 1920s to provide water for irrigation, but other relevant human activities as hunting and fishing have also been very relevant for decades. Millions of lead shots have been widespread in this wetland for more than 80 years. In 2001, a national regulation banning the use of Pb shots for hunting in Ramsar sites was approved in Spain.

In order to assess if this regulation was showing effectiveness on lead exposure in the waterfowl species inhabiting El Hondo, an appropriate biomonitoring scheme including specimens found death between 1998 and 2022 was carried out. The influence on tissue lead concentrations of many other variables were also assessed, like species, feeding behavior (divers, swimmers and flamingos), body weight, age, physical condition, cause of the death, clinical signs, and number and weight of the lead shot pellets found in the gizzard during the necropsy.

A total of 150 carcasses were necropsied and the lead concentration was quantified in samples of liver, brain and bone. Fifty-seven animals were classified as swimmers (17 mallard ducks - *Anas platyrhynchos*, 10 red-crested pochards - *Netta Rufina* and 30 marbled teals - *Marmaronetta angustirostris*), 70 as divers (30 common pochards - *Aythya ferina* and 40 white-headed ducks - *Oxyura leucocephala*) and 23 were flamingos (*Phoenicopterus roseus*).

Lead concentrations before 2001 were higher than after the ban in all tissues and in all species, but these decreases were not significant. Linear regression model studies were carried out. The models showed that the type of feeding was the factor more relevant to explain the Pb concentrations in liver and bone. However, in brain the species was the factor more explanatory. After that, the interactions in each group were assessed. Number of lead shot pellets in the gizzards was the factor able to explain the lead concentrations in the three organs.

In conclusion, Pb concentrations have decreased since 2001; however, 22 years later, the impact of lead exposure on health in waterfowl remains high, mainly in divers species (white-headed duck and common pochard) and flamingo from El Hondo Natural Park. Moreover, the number of lead shot pellets in gizzards can be used together liver concentrations to estimate the risk associated to the ingestion of lead shot pellets. Other additional measures should be considered to reduce lead exposure in these species.

#### **6.01.P-We489 Gene Loss events shaped the Chemical Defensome in Cetacea**

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Whales and dolphins hold records of contaminant accumulation, due to their ecology and life history. Large loads of environmental chemicals (e.g. persistent organic pollutants and chemicals of emergent concern) were shown to bioaccumulate in many tissues, mirroring current trends in environmental chemical build-up: a trademark of the Anthropocene. How animals deal with environmental contaminants is largely influenced by the Chemical Defensome; that is, an integrated network of genes and gene pathways that participate in metabolic detoxification and excretion of xenobiotic chemicals. Yet, the repertoire of genes related to detoxification pathways seems to have undergone evolutionary adaptations across mammalian evolution, most notably in cetaceans. Interestingly, cetaceans were shown to lack two functional xenobiotic nuclear receptors: pregnane X receptor (PXR) and constitutive androstane receptor (CAR). PXR and CAR are ligand-modulated transcription factors, activated by xenobiotic and endobiotic substances, that orchestrate the expression of drug-metabolizing enzymes and transporters. Using comparative genomics approaches, including synteny analysis and gene inactivation inference, coupled to the transcriptomic analysis of liver RNA from a double knock-out PXR/CAR mouse model, we addressed the conservation status of PXR and CAR-driven genes coding for drug-metabolizing enzymes within cetacean lineages. Gene expression analysis highlighted differentially expressed xenobiotic metabolism genes in double knock-outs. Next, four cetacean species were analysed—blue whale (*Balaenoptera musculus*), narwhal (*Monodon monoceros*), vaquita (*Phocoena sinus*) and bottlenose dolphin (*Tursiops truncatus*)—and their genomic loci of interest were compared to human (*Homo sapiens*), cow (*Bos taurus*) and hippopotamus (*Hippopotamus amphibius*), the latter representing the closest species to Cetacea. A striking reduction in the number of genes from the prototypical oxidation and conjugation families regulated by PXR/CAR was found (Cytochromes P450s, UDP-glycosyltransferases, Sulfotransferases), in agreement with previous observations with Glutathione S-transferase clusters. Our results suggest a drastic reshaping of the Chemical Defensome in cetaceans.

This work is part of the project *Whales and polar bear in a petri dish: decoding marine mammal toxicology through in vitro and in silico approaches* – *Marma-detox* funded by the Research Council of Norway, project no. 334739, 2023-2027.

#### **6.02.P Can Science Help to Respond to the Regulatory Challenge to Demonstrate the Safe Use of Chemicals for Combined Toxicity in the Environment?**

##### **6.02.P-We490 The Mixture Assessment (or Allocation) Factor as a tool for prospective mixture risk assessment: a discussion of the pros, cons and the way forward**

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Chemicals are subject to a prospective risk assessment before they enter the European market. Given that more than 20 000 chemicals are registered under REACH alone, and the increasing realisation that humans as well as the environment are constantly exposed to a complex melange of different chemicals, a major question in the context of substance-oriented regulatory frameworks such as REACH is: how can the risk of a mixture, which is typically higher than the risks of each of its individual components, be accounted for already during the registration and authorization of an individual substance? Specific, targeted mixture evaluations are usually not feasible, given that the specific evaluation of each relevant co-exposure scenario for all uses of a substance is well beyond the capacity of an individual registrant.

Therefore, the inclusion of an additional assessment factor (safety factor), termed the “Mixture Assessment Factor” (MAF), has been put forward in the European Chemicals Strategy for Sustainability (CSS), for industrial chemicals that are regulated under REACH. However, the term as used in the CSS lacks specification. As a consequence, several MAF types have been discussed in various meetings, workshops and also in the scientific literature.

This presentation will provide a brief overview of the different MAF types and then discusses their application in a regulatory context, using REACH as the prime example. Pros and cons will be analyzed with a view on the precautionary principle, the proportionality principle, cost-benefit considerations and risk acceptability.

##### **6.02.P-We491 Substance-group specific Mixture Allocation Factors (MAFs) analysis using Dutch surface waters monitoring data**



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Current regulatory chemicals risk assessment does not consider exposure of aquatic environments to unintentional mixtures, leading to potentially insufficient protection of these environments. We have evaluated the (theoretical) effects of the introduction of a generic Mixture Allocation Factor (MAF) as a possible regulatory policy measure to improve protectiveness. This evaluation is based on scenario calculations using >5.000.000 measured environmental concentrations (MECs) of 878 unique chemicals in Dutch surface waters in the period of 2013-2018. The aim was to determine:

- a) whether Dutch surface waters are sufficiently protected against mixture risks, and
- b) whether, and in how far, implementation of a generic MAF, or of substance-group specific MAFs result in improved water quality.

To this end, the exposure data was used to calculate summed Risk Coefficient Ratios (RCRs) of all mixture components, followed by scenarios in which these RCRs were reduced by increasing MAF values to lower the overall risks (methodology Rorije et al. 2022 <https://doi.org/10.1016/j.scitotenv.2022.153385>). This process was repeated for MAFs that would only act on specific substance groups. Substance groups include SVHCs, metals, industrial-, pharmaceutical- and pesticide chemicals.

The monitoring data showed that the “toxic-free environment” (defined as ‘no mixture toxicity effects are expected to occur’) is currently not present in the Netherlands, warranting measures to improve water quality. Introduction of a generic and relatively low MAF leads to relatively large improvements in water quality – “**The first blow is half the battle**”. Implementing a MAF for only one specific substance group (e.g. SVHCs) or only in one legislative framework (e.g. industrial chemicals) is far less effective. However, both a generic as well as substance-group specific MAFs can potentially help to rapidly reduce the ‘Distance-to-Target’.

In conclusion: a) monitoring data from the Netherlands shows there is a need to consider mixture exposures in ecotoxicological risk assessment, and b) a relatively small MAF, even if introduced for only a subgroup of all chemicals present in the environment, can already lead to significant improvement in environmental quality.

The data suggest that implementation of MAF is key to the ‘toxic free environment’ goal, the ‘first blow’ matters most, and growing insights in water quality as well as MAF impact can be used to refine a MAF as needed.

#### **6.02.P-We492 Comparison of mixture risk indicators in the aquatic environment**

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The environmental risk assessment of chemicals in the EU currently focuses largely on the exposure and effects of the substance or product of concern while neglecting the presence of other substances in the environment and related combined effects. In order to change that, the latest proposal by EU regulators suggests to use the mixture allocation factor (MAF) to consider the concurrent presence of other chemicals in the environment. The concept of concentration addition is applied, where the sum of the toxicity of each mixture component should not exceed the assumed safe risk quotient of 1. As a part of several PARC projects, we compare a range of MAF approaches for prospective risk assessment of mixture toxicity based on chemical monitoring datasets from European surface waters. Next to the MAF proposed by KEMI and REACH in 2021, we introduce two alternative MAF calculation methods and also plot the maximum cumulative ratio (MCR). The aim of the project is to compare the various approaches in their assumptions, the data requirements for their calculation, the calculated percentiles of the respective MAF distributions and calculated MAF values in order to evaluate their suitability and applicability to assess aquatic mixture risk.

#### **6.02.P-We493 MEED: Progress with the Metals Environmental Exposure Data Collection program to Anticipate the Challenges of the EU Zero Pollution Ambition Policy and the Chemicals Strategy for Sustainability**

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The EU Chemicals Strategy for Sustainability (CSS) is one of the main pillars of the Zero Pollution Ambition and a key building block of the Green Deal. Although the REACH revision process is delayed, the potential introduction of a Mixture Allocation Factor (MAF), to demonstrate safe use and lack of impact on ecosystems from the cocktail of chemicals exposures encountered in environmental compartments, remains a key scientific challenge for the EU industry. The MAF contributes to the overall objective of reducing *exposures of chemicals to levels that are no longer expected to be harmful to health and the environment*.

The use of metals in technologies to achieve the Green Deal objectives, like electric vehicle batteries, solar cells, windmills and electronics results in unprecedented volume increases. It is therefore crucial to demonstrate that exposure levels of emitted

metals will not increase proportionally to their uses and that the possible effects resulting from their combined exposure meet the challenge of the Zero Pollution Ambition, now and under future emission scenarios.

In anticipation, the EU metals sector designed a comprehensive “environmental exposure gathering program” (MEED), complemented by the development of scientific concepts and further test work, to measure and assess progress towards the Zero Pollution Ambition and the objectives of the EU biodiversity policy. The program runs for 3.5 years (2022- to mid-2025) and consists of 6 interlinked projects and is now halfway. Important milestones have been reached including: the identification of metals that contribute the most to the combined risks in soil and the aquatic environment, an update of today’s regional background levels for metals, a review and reappraisal of existing knowledge on metals mixtures & metals-organics mixtures interactions, the identification of testing needs to fill major gaps in the knowledge of mixture interactions, gap filling research on mixture toxicity and the design of a tiered assessment system for the local and regional biodiversity impact assessments. This poster presents the overall progress and intermediate conclusions of the MEED projects, while a detailed assessment of each of the individual milestones are presented in complementary posters.

MEED delivers a huge amount of data and relevant concepts to be used in REACH registration updates and that are key to assess progress with the Zero Pollution ambition policy of the EU in a scientific way.

#### **6.02.P-We494 Metal Mixture Effects in the Aquatic Environment: Evaluating the Potential for Refinement of the Default Mixture Allocation Factor (MAF) Based on Experimental Data**

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The European Commission calls for an investigation of the integration of combined exposure into REACH. To cover for such effects a default Mixture Allocation Factor (MAF) will be introduced, which probably will imply that the safety limit of single substances will be reduced. The implementation of a MAF will have a large impact on the environmental risk assessment of inorganics and may result in exceedances of safety limits at natural background concentrations. To anticipate the mixture correction under REACH, a dedicated research program on chronic metal mixture toxicity in the aquatic environment has been set up as part of the Metals Environment Exposure Data (MEED)-program. The current study discusses the progress made so far and gives an outlook on how the results may be potentially used to refine default MAF-setting for inorganic substances.

A comprehensive literature review of chronic metal mixture toxicity was conducted and amended with newly generated experimental data. The review showed that chronic metal mixture effects can be predicted with the standard mixture reference models, Concentration Addition (CA) and Independent Action (IA), whereby CA results in more conservative predictions compared to IA. The prediction performance of CA at low effect levels (i.e., 10% mixture effect) has been expressed as the Mixture Interaction Factor (MIF). The MIF quantifies the prediction performance of CA and is indicative of additive ( $\approx 1$ ), antagonistic ( $>1$ ) or synergistic ( $<1$ ) interactions. For the comprehensive dataset, a median MIF of 1.3 has been derived, indicating that on average CA overestimates metal mixture toxicity at these regulatory relevant low effect levels.

MAF assessment strategies have traditionally been based on the application of CA at the regulatory threshold-level (e.g., predicted no-effect concentration or 5% hazardous concentration). As such, the MAF-derivations are associated with several implicit assumptions, which cover different levels of conservatism. The MIF directly quantifies part of this conservatism, i.e. at the species-level. As such, the MIF is proposed as a factor that may be used to increase the scientific relevancy of MAF-values for inorganic substances. Future research efforts within the MEED-project will further focus on quantifying the conservatism provided by a regulatory MAF setting and developing tools for scientific refinements of the proposed mixture risk assessment methodology for inorganic substances.

#### **6.02.P-We495 The accuracy of the Concentration Addition and Independent action model to predict the toxicity of complex metal-metal mixtures to *Daphnia magna* – Is IA the better model?**

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Chemical risk assessment is predominantly designed around the regulation of single substances. However, along their lifecycle, chemicals will end up in the environment, mix with other substances and form unintentional mixtures that are not regulated by legislations like REACH. To manage these unintentional mixtures, the European Union has proposed to introduce a generic MAF (Mixture Allocation Factor) into chemicals policy. However, the magnitude of the MAF value is currently being debated.

Chemical mixtures are mainly assessed according to the concentration addition (CA) model which generally assumes that the compounds present in the mixture follow the same Mode of Action. At low effect concentrations, CA seems to overestimate metal mixture toxicity at the species level.

For complex metal-metal mixtures, we tested the hypothesis that the independent action model (IA) is in general a more accurate predictor of metal mixture toxicity than CA. Deviations of the “observed toxicity” from the “predicted toxicity” by CA can be quantified with the MIF (mixture interaction factor), which indicates either additive (MIF ≈1), synergistic (MIF<1) or antagonistic (MIF>1) interactions, relative to CA.

Our second hypothesis was that the MIF increases with an increasing number of metals present in the mixture.

Both hypotheses were tested in three large mixture experiments using *Daphnia magna*, which assessed chronic mixture effects of different combinations of Ag, As, Ba, Cd, Cr, Cu, Mn, Ni, Pb and Zn. These metals and their mixture combinations were previously selected in another study, based on their environmental relevance in European freshwater. This study also revealed that 90% of the mixture toxicity pressure to *D. magna* (expressed as sum of toxic units, STU) is driven in most freshwaters by five metals or less.

Each experiment tested simultaneously each single metal and a binary, ternary, quaternary and quinary mixture combination. All mixtures followed an equitoxic ray design and an additional quinary combination was included based on environmentally relevant mixture ratios.

Preliminary results of the first metal mixture experiment support our hypothesis that the MIF increases with an increasing number of metals in the mixtures and that IA is a more accurate predictor of metal toxicity. Once completed, this study will add valuable experimental data to the ongoing discussion on how to assess and estimate the potential risks of combined metal exposure.

#### **6.02.P-We496 A Tiered Toolbox to Assess the Impact of Metal Emissions on Biodiversity Integrating Mixture Risk Calculations, Biomonitoring and Metabarcoding – Pilot Study with a Belgian Brook**

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A key part of the Green Deal as well as environmental permitting requirements is avoiding impact of chemicals on biodiversity. Current European risk assessment methodology does not assess the risk to biodiversity directly. Therefore, it is unclear how to demonstrate “absence of effects on biodiversity”. Within the Metals Environment Exposure Data (MEED) Ecorelevance project from Eurométaux, a tiered toolbox was developed to assess the local and regional additional impact of metal emissions (i.e., independent of other stressors) on biodiversity, by: mixture risk calculations (tier I), conventional biomonitoring (tier II) and metabarcoding (tier III).

In this study, the proposed toolbox was piloted to the Kleine Nete brook in Belgium as a test case. The study site contains two metal-processing plants and sampling sites were located up-, mid- and downstream from their emission points. Measured metal concentrations were corrected for bioavailability and translated to expected (mixture) risks using available mixture risk calculation tools. Additionally, the Kleine Nete is part of the biomonitoring network of the Flemish Environmental Agency (VMM), offering the opportunity to assess the impact of the metal emissions on long historical series of biodiversity indices of macrophytes, periphyton and macroinvertebrate communities. Finally, a sampling campaign of periphyton biofilms was initiated to determine the potential impact of metals using metabarcoding based on 18s rRNA markers. Species diversity in the periphyton biofilms was used as an indicator for biodiversity effects. This case study allows to evaluate the usefulness of individual techniques to assess the local impact of metal emissions on biodiversity and investigate how the different tiers can inform each other. In the future, this toolbox aims to provide stakeholders with a flexible, tiered methodology to answer regulatory concerns about local impacts on biodiversity.

#### **6.02.P-We497 Comparison of Herbicide Mixtures at Two Catchments in Belgium**

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A large-scale monitoring study (2010 to 2013) was conducted at two Belgium catchments, Grote Kesselbeek (GKB) and Kleine Aa (KAA), in an effort to better understand herbicide mixtures in agricultural landscapes. Both sites included a measuring point at the catchment outlet and a 2nd upstream. The concentrations of 12 herbicides and 1 metabolite were assessed on a sub-daily or daily frequency. A recent publication analyses the data from the upstream GKB site using a concentration addition approach to determine measurements that exceeded the Regulatory Accepted Concentration (RAC; exceedance events) resulting in potential effects of the mixture on algae and macrophytes. These exceedance events coincided with seasonal influences, and low rainfall during spring/summer 2011 correlated with a highly reduced number of these events. However, the most significant impact on the exceedance events was found to be a stewardship program that was implemented following an initial 8-month baseline measuring period. The stewardship directed farmers to use more advanced farming techniques, avoid spillages and other point sources. Exceedance events were reduced by more than half for algae and by approx. 10 times for macrophytes from the year the stewardship was established onwards. These findings show how local measures reducing point sources are highly effective and potentially more so than desk-based approaches focusing on diffuse sources.

Data were further analysed in comparison to the measuring point at the outlet of the GKB catchment, and to the data from both measuring points of the KAA catchment. These catchments differed in their size, land use, erosion likelihood and farms in the surrounding area. The KAA catchment is approx. 10 times larger than the GKB catchment (10K and 950 ha, respectively). Correspondingly, 950 farmers cultivate the land at KAA (120 farmers at GKB) and the KAA %farmland was found to be lower. Due to the comparably large size of the KAA catchment, no stewardship was conducted as part of the monitoring program.

Initial findings show that the quantity of exceedance events differs significantly between sites, with exceedance events being more prevalent at the GKB catchment compared to the KAA catchment. Although less prevalent, the events at the KAA site were found to be more often driven by true mixtures. The poster will investigate the drivers for mixtures beyond the RAC and discuss concepts and ideas to reduce these.

#### **6.02.P-We498 Evaluating the Effects of Metal Mixtures on Freshwater Invertebrate Communities**

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Environmental Quality Standards (EQS) accounting for metal bioavailability, beyond hardness corrections, have now been developed and implemented throughout Europe for copper, lead, nickel and zinc for several years. Standards for lead and nickel were set on a Europe wide basis and those for copper and zinc have been set by individual member states. Available evidence suggests that ecological communities are not compromised to an identifiable extent at locations that comply with these EQS values, although the variability associated with ecological data means that there is some uncertainty associated with these findings. There is increasing regulatory concern at a European level about the possible effects of the mixtures of multiple contaminants in the environment. This is based on the concern that multiple contaminants, all of which are present at levels that are safe based on exposures to the individual substance alone, could result in adverse effects due to the additional stress resulting from multiple exposures. Copper, lead, nickel, and zinc are all commonly regulated metals for which robust EQS values are available that can be normalised for bioavailability. This study evaluates whether EQS derived for individual metals based on single substance exposures are sufficiently protective in the environment where a mixture of different metals may be present. Analyses of the response of the benthic macroinvertebrate community to exposures of both the individual metals and their overall mixtures were analysed based on both EQR N-Taxa, and EQR ASPT. A decline in ecological community quality, as EQR N-Taxa, is seen for some, but not all, of the individual metals considered and for the overall mixture. Single metal derived EQS values are protective of ecological quality, as expressed by EQR N-Taxa, although high metal exposures are associated with a reduced maximum achievable ecological quality based on EQR N-Taxa. Although high mixture exposures are also associated with reduced ecological quality this is only associated with very high total RCR values.

#### **6.02.P-We499 Systemic Toxicity Screening of Mixture Chemicals in Consumer Products in Korea: Case Study on Cleaning Products**

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In consumer products, diverse individual chemicals are mixed and co-exposed to consumers, underscoring the imperative for comprehending the mixture toxicity in the varied components employed across these products. Therefore, we conducted a case study with tiered approach to screen the systemic toxicity of binary combinations of chemicals used in Korean cleaning products. In step 1, we collected information on 5,171 cleaning products from the Korean consumer product database, Ecolife, and extracted 811 chemicals used as main ingredients. In step 2, we selected 52 chemicals, including the top 20 most frequently used chemicals and 32 chemicals under the government regulation. In Step 3, information on the hazards of individual chemicals was gathered from the reports published under K-REACH, EU REACH, US FIFRA, and EU-BPR. And the chemicals were grouped by their hazards such as respiratory, endocrine, and neurotoxicity. Subsequently, binary combinations of chemicals showing common hazard were prioritized based on their frequency of use in products. Additionally, we enhanced the evidence for binary combinations by comparing them with combinations obtained using association rules. In Step 4, we evaluated the mixture toxicity of the selected combinations through cytotoxicity screening. In this study, we identified binary combinations of chemicals that could cause mixture toxicity in cleaning products. This study highlights the blinding point and persistent challenges concerning the mixture toxicity of chemicals contained in products used in Korea. For further study, we plan to evaluate the mixture toxicity of these combinations in a mechanism-oriented approach by applying an integrated testing strategy based on adverse outcome pathway framework.

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Keywords: Korean Consumer Product Database, Cleaning Products, Association Rules, Mixture Toxicity, Toxicity Screening

## 6.02.P-We500 Impact of exposure assumptions on the size of mixture assessment factors required to protect environmental receptors

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A Mixture Assessment Factor (MAF) is an additional safety factor proposed for use in the setting of ecological standards for individual chemicals. The MAF is intended to protect receptors from the cumulative effects of concurrent exposures to multiple chemicals. The Swedish Chemicals Agency (KEMI, 2021) recently proposed a numerical approach to derive the values of MAFs required for mixtures of chemicals measured in surface waters (the KEMI MAF). Although the KEMI MAF is an improvement over earlier approaches, it generates large estimates of MAF values because of assumptions made concerning certain inputs to the MAF calculations. The affected inputs include: the number of chemicals that reach an individual receptor; the allocation of the chemicals into mechanism-based assessment groups; the variation in combined exposures over time for an individual receptor; and the concentrations of chemicals that will occur under future controls of individual chemicals. In this work, we explore the impact of alternative assumptions for these inputs using methodologies developed in a recent publication (Price and Junghans 2023). A range of MAF values are generated for the mixtures reported by multiple large-scale surveys of EU surface waters. These values demonstrate how alternative assumptions for the inputs have significant impacts on the size of MAFs derived using the KEMI MAF approach.

## 6.02.P-We501 Addressing the Impact of the Mixture Allocation Factor (MAF) on Environmental Risk Assessment: Refining Regulatory Exposure Predictions Using Spatial Data and Modelling Approaches

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Under the Chemical Strategy for Sustainability (CSS), the European Commission has proposed the use of a mixture allocation factor (MAF) to address uncertainties and risks associated with unintentional chemical mixtures in the environment. Currently it is thought that a MAF may need to be applied to all single chemical registrations for the higher tonnage bands. It is expected that additional exposure and/or effect data generation will be needed for the risk assessment, and in some cases further risk management measures may be required to ensure environmental safety. The European Union System for the Evaluation of Substances (EUSES), also accessed via the CHESAR v1 tool is the standard model to determine exposure in support of chemical safety assessments (CSAs) under REACH. In the spirit of continuously refining safety assessments and models, we envisage an opportunity to update exposure model background data in EUSES/CHESAR in line with the evolution of wastewater treatment infrastructure in EU, and availability of better spatial datasets. This would ensure that regulatory modelling remains aligned with most updated data available and the situation in the field. In this work we explore field data and spatially explicit modelling approaches, some of which are probabilistic, in order to compare them with the current EUSES default parameters. We developed a spatially explicit model which applied the EUSES PEC calculation to estimate environmental concentrations at freshwater discharge locations of almost 22,000 European Urban Wastewater Treatment Plants (UWWTPs). Specifically, using publicly available data, we examined per capita water use, UWWTP connectivity and local UWWTP dilution factors to generate EU-wide distributions and to place these in context with default EUSES values. We demonstrate that spatially explicit exposure models are feasible for use in regulatory modelling and can give more realistic predicted environmental concentrations (PECs). Case studies have been performed comparing PEC<sub>local</sub> values for freshwater using CHESAR v1 including both the current default data and the spatial approach. We expect that higher tier exposure data will be increasingly needed to ascertain that risk quotients remain acceptable even with a MAF applied.

## 6.02.P-We502 Monitoring risks of chemical mixtures in humans

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Human beings are constantly exposed to chemical mixtures from various sources. While the safety of intentional mixtures is addressed in the chemical risk assessment under the EU legislation, the existence of unintentional mixtures has not yet been properly taken into account. The Chemical Strategy for Sustainability aims to strengthen the EU's policy in this area, including the development of indicators to analyse trends and progress towards policy objectives. Human biomonitoring (HBM) data offers an aggregated exposure insight that combines all exposure sources, independent of the pathway (exposure via consumer use/articles, diet, and environment) and policy specific domain. Here we propose a human health risk indicator based on HBM data to explore temporal trends in the risk from chemical mixtures. The indicator uses HBM data available in the Information Platform for Chemical Monitoring (IPCHEM), coming from studies such as Democophes and HBM4EU that investigated the presence of several chemicals in the general European population in the period spanning from 2007 to 2021. Chemicals were prioritised for HBM based on their health concern and EU policy relevance. The indicator is expressed as a ratio of internal exposure levels and the established health-based guidance values. The sum of ratios indicates a potential health risk associated with the monitored set of chemicals, whereas a sum greater than 1 indicates that further refinements are needed to confirm or exclude risks. The indicator only includes chemicals that were measured in both reference periods (P1:2007-2014 and P2:2014-2021) and for which internal reference values exist, yielding a total of 15 chemicals. Seven substances, in particular, are the main drivers of the risk: bisphenol A, the perfluorinated chemicals PFOS and PFOA, arsenic, cadmium and two

phthalates (monobutyl phthalate and monoisobutyl phthalate). The comparison of risk ratios between the two time periods suggests a decreasing trend in risk. However, we are aware that differences in study set ups, including the age of participating subjects and the variation in the EU coverage, could have had a large influence on the overall trend. The future European HBM data that is being generated under the Partnership for the Assessment of Risks from Chemicals (PARC) and that will represent the next time point in our indicator will strengthen our ability to conclude on trends.

#### **6.02.P-We503 Review of the human mixture risk assessment cases: Research needs for scientific evidences to select mixture allocation factors in human MRA**

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Humans can be exposed to numerous chemical mixtures that might impact health, and various mixture risk characterization methodologies have been used. But still, in regulatory silos, mixture risk assessment (MRA) is not included in detail and thus not to be used for mixture risk management. To obtain real-world risks for humans, MRA should be applied as soon as possible, and providing scientific evidence is urgent. For this reason, this study aimed to gain future insights into MRA methodology development. In the first part, to determine the most frequently used MRA methods and data gaps, peer-reviewed literature was searched, and 39 studies were accepted for all eligibility criteria for study objectives. Currently, component-based and concentration-addition approaches were used in most studies, and risk characterization was conducted using Hazard Index (HI) methods most frequently. Still, no methodology considers interaction among chemicals. However, if the synergistic interaction exists among substances in the mixture, the mixture risk might be underestimated. Development of the MRA methods that consider chemical-by-chemical interaction is needed and considering the mixture reference dose (RfD<sub>mix</sub>) method is suggested here. In the second part, we tried to provide the weight of evidence for the mixture allocation factor (MAF). Recently, because of insufficient data for toxicity and exposure to mixtures, MAF is considered a rational alternative to treat mixture risk even though its pragmatism. Research needs and evidence might be provided by our case study using KoNEHS 3<sup>rd</sup> biomonitoring data, showing HI>1 in median and 95<sup>th</sup> percentile exposure scenarios for 11 kinds of non-persistent endocrine disrupting chemicals (EDCs). Our results demonstrated that health risks from unintentional mixtures are already at risk, and a 3- to 10-times lower limit for risk characterization can be suggested in the current exposure level. We expect our suggestions and case study to help make reasonable management options for total chemical exposures and apply options in chemical legislation.

#### **6.02.P-We504 Offshore Chemical Regulatory System & Risk Assessment**

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Protection of the marine environment from the discharge of chemicals used offshore is of global importance. Sustainable operations in the oil and gas sector requires a regulatory framework ensuring protection of the ecosystem and prevention of deleterious environmental impact.

Chemicals' use and discharge for petroleum exploration activities in the OSPAR region require chemicals to be registered via the OSPAR's Harmonised Mandatory Control System (HMCS). The objective of the HMCS is to protect the marine environment by identifying those chemicals used in offshore oil and gas operations with the potential for causing an adverse environmental impact and restricting their use and discharge to the sea. Thus, for the chemical supply industry there is pressure to develop products that comply and fulfil technical performance and environmental risk criteria, Operators' (oil companies) requirements are more linked to ensure optimum operational performance.

On the environmental regulatory front Recommendation 2012/5 requires Contracting Parties to implement a risk-based approach (RBA) for the management of produced water discharges from offshore installations. RBA utilises a stepwise screening tiered approach involving either whole effluent toxicity and/or substance -based approach with use of dispersion modelling to establish if chemical discharges are likely to result in adverse effects to the marine environment.

Does this approach regarding methodology and regulatory framework criteria address the evaluation of potential combined effects of chemicals? Does the regulatory implementation require field monitoring to assess the impact on the ecosystem?

#### **6.02.P-We505 SAFETY ASSESSMENT OF FLOW BATTERY ELECTROLYTES IN NEW ALTERNATIVE MODELS SUCH AS ZEBRAFISH, CRUSTACEAN AND MICROALGAE**

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Redox flow batteries are an emerging technology for medium and large-scale stationary energy storage. the safety and human toxicity aspects of the electrolytes in redox flow batteries as well as their corresponding environmental impacts are poorly documented. This proposal seeks to perform different bioassays to assess the toxicological and ecotoxicological screening of several quinone based electrolyte systems. The teratogenic potential and any goitrogenic properties of the items were assessed by the developmental defects and thyroid disruption in zebrafish embryos. The ecotoxicological profile was done by applying the *Daphnia magna* immobilization assay and the microalgae *Raphidocelis subcapitata* growth inhibition test. These last two assays are based on the OECD 202 and 201 guidelines and comprise a simplified and more efficient assay design, for further sustainability.

The results obtained for their teratogenic analysis reveal that some of the quinones were classified as toxic for zebrafish embryos and showed high percentages of affectations and mortality at the highest tested concentrations. However, one quinone redox active component was classified as not toxic since the teratogenic index value was not estimated and no more than 20% of affected embryos were observed under the tested conditions. No implication for any goitrogenic effect was assessed for the four tested electrolytes with the thyroid disruption assay. No changes in fluorescence intensity were detected after exposure to four compounds, all values being similar to the control. Two electrolytes were highly toxic to *Daphnia magna* since the differences in the percentage of immobilization between treated and control groups were higher than 20% at very low concentration ranges. Two other electrolytes were not toxic to *daphnia* in the tested concentration range since no or very low immobilization was detected after 48h of exposure. For the microalgae assays of the four electrolytes, a complete dose-response curve was calculated comprising 0 – 100% effect. All four test items induced toxicity with EC50 values in a similar range.

There are differences in the (eco-) toxicological profile between the test items. The newly designed quinones showed mostly less human toxicity and ecotoxicological potential across all assays. We can conclude the bioassays proposed in this work can properly assess the (eco) toxicological profile of different electrolytes more sustainably and in a dose-effective range.

## **6.02.PC Can Science Help to Respond to the Regulatory Challenge to Demonstrate the Safe Use of Chemicals for Combined Toxicity in the Environment?**

### **6.03 Combining Prospective and Retrospective Soil Risk Assessment - From Predicted Risks Towards Holistic Approaches by Integrating Monitoring Results**

#### **6.03.T-01 Concepts to define a “normal operating range (NOR)” for soil biological systems**

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The definition of a normal operating range (NOR) would be a very valuable tool to classify the statistical and biological results of species and population soil testing systems at different tiers. In the EFSA opinion on recovery an overarching definition for a NOR is described as “The acceptable range of values of a measurement endpoint that is normally observed during a predefined period for a reference population, community, ecosystem or process” (EFSA journal 2016:14(2):4313).

The definition of NORs in semi-field and field tests requires different scales of observation and a tailored application range of the NOR output. A proposal to basically define the NOR of a single observation timepoint in a field test for a specific taxon could be: “The NOR is defined as the percentage by which the abundances deviate from the mean control values for a given taxon at one observation point. The NOR includes all values of this taxon between a defined percentile range (NOR90 includes all values between the 5<sup>th</sup> and 95<sup>th</sup> percentile of all values for a given taxon)”. This NOR90 represents the statistical significance level of  $\alpha=0.1$  and can help to better classify the statistical output for this taxon in the subsequent observation timepoint.

The evaluation of Collembola field tests shows a potentially large impact resulting from a large variability in abundances for certain timepoints and the hierarchical level on the resulting NOR90 output. For e.g. *Lepidocyrtus cyaneus/L. violaceus* the NOR90 in autumn is calculated to be 95%, whereas for the family Entomobryidae (including the *Lepidocyrtus* species) a much lower NOR90 value of about 56% can be observed.

This basic NOR-definition can also be extended to other scales of observation. In a single 1-year soil field study such scales could be NORs defined by several observation timepoints in the same season (leading to a higher temporal resolution) or a time-dependent NOR through the whole study period. For soil monitoring purposes, NORs derived from a range of field tests and the inclusion of additional data derived from suitable publications and databases (e.g. Edaphobase, Collembola.org) could be relevant to define baseline scenarios for the investigated soil organism groups.

#### **6.03.T-02 Applying the Pesticide Load Index to characterize ecotoxicological impact from pesticide use in the EU**

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Improving the sustainable of agriculture requires an advanced assessment of the ecological impacts of pesticides. Our study applies the Pesticide Load Index (PLI), already been used in Denmark and in England, to quantify pesticide risks to environmental health and biodiversity across the European Union. This could help to further integrate ecological considerations into assessments of plant protection products. The PLI is defined as the sum of the amount of applied pesticide active substances (AS) divided by the toxicity to non-target-organisms, including birds, mammals, and beneficial insects like bees and natural enemies of pests.

Our methodology bridges the gap between ecological health and pesticide risk assessment using three extensive data sets. The first data set includes EU-wide estimations of AS emissions at a 1km resolution. The second data set provides detailed AS use in France, including crop types. The third, from the Pesticide Properties DataBase, assesses ecological risks by comparing pesticide loads with ecotoxicological values. By integrating acute toxicity, chronic toxicity, and environmental fate, we gain a deeper understanding of possible pesticide impacts. Acute toxicity indicates short-term effects, while chronic toxicity addresses long-term consequences. Environmental Fate Load examines pesticides' behavior and transformation in the environment. The resulting granular PLI maps are essential for identifying areas with high ecotoxic levels, informing where additional risk mitigation measures are necessary.

In conclusion, the study's outcomes offers new insights into the ecotoxicological impacts of European Union pesticide risk distribution, equipping policy-makers and farmers with spatially explicit data to tailor strategies for biodiversity and ecosystem conservation. This can be used to inform and monitor the EU's Farm to Fork and Biodiversity strategies, particularly the goal of reducing pesticide use and risk by 50% by 2030. In addition, the comparison between crops, regions, and EU Member States could help in developing further protective and scientifically sound regulatory frameworks to bring potential economic benefits, increase agricultural resilience and reduce pesticide dependence by through nature-based (such as natural pest control and pollination) mechanisms.

### **6.03.T-03 Plant Protection Product Residues in Agricultural Soils Across Europe, and the Effect of the Farming System on the Soil Microbiome**

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As more and more studies show, the occurrence of mixtures of plant protection products (PPP) in soil is more often the rule than the exception. The effects of such mixtures on soil life are not completely understood yet. A “cocktail effect” of more than one PPP residue could lead to a higher chemical pressure on the soils and consequently soil life, which could reduce its capability to provide essential ecosystem services. In the SPRINT project, 101 organically (OF) and 100 conventionally (CF) managed fields were sampled across 10 European countries. The soil samples, collected at the middle of the 2021 growing season were analysed for 192 different PPP residues, and DNA was isolated for microbiome analysis. During the sampling campaign questionnaires about amongst others, the PPP applications were conducted. The application records were used to predict the environmental concentrations (PEC) of the PPPs in soil at sampling time. These PECs were compared to the measured environmental concentrations (MEC). DNA isolates were used for amplicon sequencing and to analyse differences in microbial composition (e.g., total, nutrient cycles) between fields under different farming systems. Additionally, PPPs with the same mode of action were clustered and the effects of these groups on the microbial composition were analysed. In total 101 different PPP residues were detected. At least one PPP residue was present in 99% of CF fields and 95% of OF fields, and mixtures of PPP residues have been detected in 96% of CF and 79% of OF fields. These high detection frequencies especially in OF fields are in part alleageable by organochlorine pesticides which have been long banned but persist since then. Without these there are still PPP residues found in 95% of CF fields but only in 64.4% of OF fields. Of the PPPs analysed and applied in the growing season before sampling, 51.5% have been detected 79% of PPP residues detected were not applied in the 2021 growing season. The PECs calculated did not fit well with the MEC, with both under and over-estimations of the environmental concentration. The first results of the analysis of the sequencing data show that the country is the main driver behind microbial composition. Indications are that the microbial (alpha-)diversity is not affected significantly by the farming system, but the composition is. Further analysis of the effect of different pesticide groups, and on different groups of microorganisms will be conducted.

### **6.03.T-04 Evaluation of the Ecological Risk of Pesticides Residues from the European LUCAS Soil Monitoring 2018 Survey**

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The 2018 LUCAS pesticide survey provides a EU-scale assessment of 118 pesticide residues in 3,473 soil sites. This study responds to the policy need to develop risk-based indicators for pesticides in the environment. Two mixture risk indicators are presented for soil, based respectively on the lowest and the median of available no observed effect concentration (NOEC<sub>soil,min</sub> and NOEC<sub>soil,median</sub>) from publicly available toxicity datasets. Two further indicators were developed based on the corresponding equilibrium concentration in the aqueous phase and the aquatic toxicity data, available as species sensitivity distributions.

The mixture risk indicator based on the NOEC<sub>soil,min</sub> exceeds 1 in 1.7 % of sites and 0.1 in 22% of sites. The insecticides imidacloprid and chlorpyrifos and the fungicides epoxiconazole and boscalid are the largest contributors to the overall risk. At each site, one or few substances drive the mixture risk. Modes of actions most likely associated to mixture effects include modulation of acetylcholine metabolism (neonicotinoids and organophosphate substances) and sterol biosynthesis inhibition



(triazole fungicides). Several pesticides driving the risk have been phased-out since 2018. Following LUCAS surveys will determine the effectiveness of substance-specific risk management and the overall progress towards risk reduction targets established by EU and UN policies. Newly generated data and knowledge shall stimulate needed future research on pesticides, soil health and biodiversity protection.

### 6.03.P Combining Prospective and Retrospective Soil Risk Assessment - From Predicted Risks Towards Holistic Approaches by Integrating Monitoring Results

#### 6.03.P-We506 Disentangling uncertainties in the Environmental Risk Assessment of pesticides for soil organisms – UNCERTAIN project

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Several uncertainties associated with the Environmental Risk Assessment (ERA) process have not been taken into account, either due to unawareness or a lack of data and/or tools to handle them. European Food Safety Authority (EFSA) acknowledges these uncertainties and recently provided guidance on how to handle them in various scientific assessments, like in the ERA of pesticides. The UNCERTAIN project aimed to identify and quantify sources of uncertainties (SOU) that may occur in the ERA of pesticides, especially in the soil compartment. Moreover, new data was produced by carrying out a set of laboratory tests using a targeted combination of soils, test species and substances to close knowledge gaps on uncertainties that have not been adequately addressed by the data available in regulatory databases and the literature. These new data aimed to understand and describe how experimental results (subsequently used in risk and fate modelling) may differently affect the outcome of an ERA. Lastly, predominant and pre-defined SOUs were analysed using Bayesian network models combining results generated in the project and data from publicly available and protected databases. A total of 53 different SOU were identified but partly overlapped in different categories and only 14 were assumed to be quantifiable by adequate statistical methods, given the availability of suitable data. The new data produced showed that using artificial soils in ecotoxicity testing with soil invertebrates can be insufficiently protective in toxicity evaluation of chemicals. Data analyses of quantifiable single SOU did not suggest that including soil characteristics other than organic carbon (OC) content would significantly increase the variation explained. On the other hand, there was some evidence that models combining soil characteristics and chemical properties of test substances might be able to explain a substantial fraction of the variation in test outcomes not explained by OC percentage on its own. The current assessment factor (AF) of five for extrapolation of earthworm toxicity data from the laboratory to the field should be reconsidered to cover all the relevant uncertainties thus enabling an adequately protective ERA. For mesofauna, more data (pairs of laboratory and field study outcomes) are needed to reduce uncertainty about the extrapolation.

#### 6.03.P-We507 Ecotoxicological tests for assessing single and mixture effects of antibiotics and copper

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Antibiotics (ABs) are widely used to treat human and veterinary infections. Biosolids and manure, used as fertilizers in agriculture, can contain AB residues and can be a source of contamination for soil and water. Copper (Cu) can be found in co-presence with AB residues in agroecosystems due to its common addition in cattle feeds and in antifungal products to improve the agricultural productivity. Consequently, in real contamination scenarios, several types of pollutants can be present and can interact in different ways, including worsen or mask toxic effects of single substances. For this reason, it is crucial to evaluate mixture effects on different organisms and environmental matrices.

In this context, ecotoxicological tests with *Aliivibrio fischeri* were performed to assess single and mixture effects of Sulfamethoxazole (SMX), Ciprofloxacin (CIP), Chlortetracycline (CTC) and copper sulphate (Cu). Predicted toxicity values were calculated in accordance with the Concentration Addition model. Finally, water extracts from a soil co-contaminated by the same AB mixture and Cu, were tested for evaluating the overall effects in a real environmental matrix.

CTC resulted the most toxic AB for *A. fischeri* ( $EC_{50}=3.64$  mg/L), followed by CIP ( $EC_{50}=96.93$  mg/L) and SMX ( $EC_{50}=194.06$  mg/L), while Cu was the most toxic compound ( $EC_{50}=0.78$  mg/L). Comparing binary mixture measured and predicted toxicity values, SMX+Cu showed an additive effect, CIP+Cu a synergic effect and, interestingly, CTC+Cu an antagonistic effect. The other mixtures (ternary with the three ABs and quaternary with all the substances) showed additive effects, except for SMX+CIP+CTC that displayed an antagonistic effect.

Soil water extracts did not show toxicity, highlighting the possible buffering capacity of the soil used in these experiments. Further tests with *Lepidium sativum* and *Daphnia magna* were performed, and the results obtained were compared and discussed with those of *A. fischeri*.

Combined effect results can be due to different complexation processes between ABs and Cu that can mask or increase effects of the single chemical. The study of substance interactions in environmental matrices is a present and future challenge. This work represents a contribute to the research gap in the complex framework of mixture effect evaluations in a real environmental context.

### **6.03.P-We508 MICROSOIL - Investigation of Alternative Test Methods to Correctly Assess the Impact of Plant Protection Products, Biocides and Pharmaceuticals on Soil Microorganisms**

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One aspect in the risk assessment of e.g. plant protection products (PPP) is the evaluation if one of the most important soil functions, the N-transformation (OECD 216) of soil microorganisms, is affected. However, focussing on one central function of the microbial community is not sufficient to determine the impact on soil microorganisms. The MICROSOIL project aims to identify sensitive alternative test methods to determine the effect of e.g. PPP on the microbial community. For this purpose, the MicroResp™ test system (substrat induced respiration with various substrates), the ISO 20130 (exoenzymatic activities) and the ISO 15685 (ammonium oxidizing bacteria) were selected as alternative test systems. In addition, based on the recommendations of EFSA (2017), the fungal group was included, considering arbuscular mycorrhizal fungi (AMF) as a sensitive group.

The spore germination test (ISO 10832) will be used to evaluate the effect of PPPs, a biocide and a veterinary pharmaceutical on *Funneliformis mosseae*. This presentation aims to describe the process of i) establishing a culture of *F. mosseae*, ii) adapting the test setup outlined in the ISO 10832 for natural soils and iii) investigating the effect of six different test substances on the spore germination.

A culture of *F. mosseae* was successfully established using parsley as the symbiotic partner. Once sufficient spores were available, different natural soils (RefeSol 02A, RefeSol 04A, Lufa 2.1) were investigated as an alternative to artificial soil and sand suggested by ISO 10832. As the required germination rate was not reached with the natural soils, test conditions (e.g. the soil water content) were adapted. The reduction of the water holding capacity to 50 % was necessary to reach a sufficient germination rate. For Lufa 2.1, the required germination rate was not reached, which might be due to the low pH (< 5). RefeSol 02A (pH = 6.8; sand 79.7 %; silt 14.9 %; clay 5.4 %; soil texture: silt loam) showed a sufficient germination rate and sensitivity was validated using the reference substance benlate (1 mg/kg and 10 mg/kg). As a first fungicide tebuconazole (1 mg/kg and 10 mg/kg) was tested, but no significant inhibition was observed in the tested concentrations. Now, the effect of different other substances on the spore germination of *F. mosseae* will be tested using RefeSol 02A and likely another natural soil.

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### **6.03.P-We509 Exploring the Biodiversity of Brazilian Soils: A Review on Earthworms, Enchytraeids, Collembolans, and Soil Mites**

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Brazil, having approximately 846 million ha of land, faces challenges in managing its diverse environmental landscape, ranging from equatorial rainforests in the north to temperate coniferous forests in the south. Farming activities occupy around 60% of the total area. The soil ecosystems in Brazil are susceptible to changes in land use and environmental conditions. Key soil functions, including organic matter (OM) degradation, nutrient cycling, and water infiltration, are dependent on soil organisms. Their diversity, abundance, and seasonality are influenced by various abiotic factors, such as land use, rainfall, humidity, and temperature. The development of Specific Protection Goals (SPGs) within the framework of environmental risk assessment for pesticides becomes crucial, considering their 'Normal Operating Range' and functions in agricultural ecosystems. We conducted a literature review on the biodiversity of earthworms, enchytraeids, collembola, and soil mites in Brazil. Our aim was to evaluate their diversity and abundance across Brazil in relation to various abiotic factors. We synthesised soil biodiversity data from more than 120 field studies up to June 2023. From the retrieved publications, data related to study location, dates, sampling, soil characteristics, agricultural practices, organisms and density, were compiled in a database. We evaluated the average density of organisms vs the following factors: season of the year (rainy vs dry), OM %, soil pH and influence of tillage. Most of the ecological studies are on soil mites (> 40% of the studies), followed by earthworms, collembolans, and enchytraeids. Tillage has an impact on mesofauna density across the four groups of organisms, with a reduction in average density of collembolans and soil mites in the dry vs the rainy season. In relation to pH, Collembola and soil mite densities were higher at certain pH values, while earthworm densities were not strongly influenced by pH soil values. Other influencing factors will be presented.

Despite an increase of reported data on the abundance and diversity of soil invertebrates, our data underscores the deficiency in basic information regarding the occurrence and ecology of these organisms. This emphasizes the necessity for global

collaboration to foster a comprehensive understanding of soil biodiversity in Brazil. A better understanding of the Normal Operating range is crucial to bring potential effects of pesticides into agro-ecological context.

### **6.03.P-We510 Case Study on Soil Organism Species Richness and Abundance on Agricultural Sites in Germany**

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Risk assessment for in-soil organisms opens the possibility to conduct field studies as a refinement option if an unacceptable risk was indicated at the first step of the assessment. These higher tier field studies are conducted with field communities of soil organisms. Whereby a guideline is available for earthworms, no official specific test guideline for field studies with collembola or mites is available up to date. Information about expected values for abundance and diversity of natural communities of soil organisms has been gathered but evaluation of the data for environmental risk assessment questions are rare. Therefore, the variability of tested communities may be high and effects of pesticides on those communities might be dependent on the chosen site for the experiments. To gain a better understanding on the differences in natural communities across agricultural land and to further facilitate the development of a test guideline for higher field studies, we compiled a large dataset of species data and land use types across Germany.

Data on field studies with soil organisms submitted for the authorisation process of plant protection products in Germany was gathered from the internal UBA-database ICS. Moreover, the data base Edaphobase was used to gather and evaluate data on collembola, mites and earthworms on corresponding sampling sites. In a first step, sampling sites were grouped based on land use type. In a second step, grouped data was evaluated to identify preliminary expectations regarding species richness and abundance for soil organisms, acknowledging the land use type.

Based on the available data, we found significant differences in species richness between different land use types for earthworms and collembola. In soils on intense farmlands were significant less earthworm species present compared to extensive farmlands or grasslands.

Our analysis provides further insights in the differences of soil organism species diversity in agricultural fields. We aim at a better understanding of the prerequisites for field studies and provide data to support a harmonized assessment of expected soil communities in the field.

### **6.03.P-We511 The role of soil monitoring for the regulation of chemicals**

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Plant protection products (PPPs) are applied in large quantities [1], mostly through multiple applications [2] on agricultural land. Depending on the respective soil and active substance properties they may accumulate and remain in soils for years. In combination with other stressors, soil pollution can lead to a decline in soil biodiversity, which accounts for a large proportion of overall biodiversity and is a central building block and driver for soil fertility and other soil functions [3]. However, the overall soil contamination due to residues of PPPs in agricultural soils is largely unknown and cannot be compared with the state of knowledge on PPP residues in surface or groundwater.

The risk assessment of PPPs does not take into account possible pre-contamination of soils as well as an accumulation of substances [4], although it is known that soils can act as a sink for pollutant accumulations. The consequences of multiple and sequential PPP exposures from fresh and long lasting residues for non-target organisms, terrestrial biodiversity and the ecosystem are not sufficiently understood. So far, it has not been clarified to what extent this deficit leads to an underestimation of the risks in the authorisation procedures.

This study aims to define ‘soil monitoring’ with the focus on the risk assessment for plant protection products and gathers information on different kinds of monitorings within the regulatory approach. It compiles a subset of existing surveys in Germany and Europe and suggests further needs for the connection of monitoring programs with the risk assessment for PPP. Moreover, open questions regarding the conduction and design of monitoring programs are raised and the connection with strategies, regulations and directives within the green deal are made. The aim of this study is to draft the way to solve regulatory challenges and support the goals of the green deal as well as the soil monitoring and resilience law, aiming to reach 100 % healthy soils by 2030.

[1] Eurostat 2023. <https://ec.europa.eu/eurostat> [2] Knillmann et al. 2021. Environmental risks of pesticides between forecast and reality: How reliable are results of the environmental risk assessment for individual products in the light of agricultural practice)? [3] FAO, 2020. State of knowledge of soil biodiversity -Status, challenges and potentialities’. [4] Frische et al. 2018. 5-Point programme for sustainable plant protection. Environ. Sci. Eur. 30, 8 (2018).

### **6.03.P-We512 Retrospective pesticide mixture risk assessment for agricultural soils**

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Plant protection products (PPP) are widely applied in agricultural fields for crop protection with potential accumulation in the

soil. As a result from the serial application or slow degradation of PPP, the soil and organisms therein may be exposed to a complex mixture of PPP residues. Research on the predictability of mixture toxicity as well as retrospective risk assessments have mainly focused on the aquatic compartment so far. In recent years the soil compartment comes more into focus as can be seen from the proposal for an EU directive on Soil Monitoring and Resilience. Also several monitoring studies have been done on PPP residues in agricultural soils. For an assessment of the risk of these residues on soil organisms mixture toxicity has to be considered. Based on a review on different mixture risk assessment concepts with the focus on soils we present an approach for retrospective mixture risk assessment for PPP residues on soil organisms. Main challenges and uncertainties are discussed.

### **6.03.P-We513 Chemical, Ecotoxicological And Ecological Indicators In Risk Assessment Of The Agricultural Area Long-Term Contaminated With Organic Pollutants**

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Soils are multifunctional non-renewable resources providing goods and services essential for ecosystems and human life. Agricultural land is often located close to highly urbanised/industrialised areas and can be exposed to emissions of various organic and inorganic pollutants. As a consequence, soil quality can deteriorate and there is a risk of contaminants entering the food chain. Thus, risk assessment in areas particularly exposed to chemical degradation is still a big challenge. Most studies on environmental risk assessment have only analysed total pollutant concentrations and used chemical risk indexes, e.g. hazard quotient, toxic units or pollution load index, to assess the level of risk. Such approach does not provide information on the bioavailability and toxicity of chemicals and often leads to an overestimation of risk. Therefore, in our study we used an interdisciplinary approach whereby the final conclusions about the risk in a given area were based on the integration of detailed data from chemical, ecotoxicological and ecological analysis.

The study was conducted in the agricultural area exposed to the long-time contamination, located in the South-West part of Upper Silesia region in Poland. Chemical measurements comprised both total and bioavailable polycyclic aromatic hydrocarbons (PAHs) content. A battery of bio-assays describing effects on soil retention and habitat function was used for ecotoxicity testing, and ecological indicators included enzymatic activity, soil respiration, microbial biomass, carbon mineralisation and nitrification.

Environmental risk index ranged from 0.19 – 0.94 and was mainly due to high values of chemical risk index, while ecotoxicological and ecological results indicated no or low risk. The majority of the area (almost 90%) had acceptable risk levels, and very high unacceptable risk (EnvRI 0.77–0.94) was only at three sampling sites. Our study revealed that integration of data on the organism's response and ecological indicators into evaluation provide realistic assessment and allows for delineating the area which needs additional action (further monitoring or remediation).

### **6.03.P-We514 ARAGORN: Achieving Remediation And Governing Restoration of contaminated soils Now**

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Announcing the Horizon Europe project ARAGORN:

Soil contamination is a severe hazard to humans and the environment. We propose Achieving Remediation And GOVERning Restoration of contaminated soils Now (ARAGORN) through development and implementation of a complete framework. The framework starts from the identification of contaminated sites and presents a decision-making tree to identify remediation and restoration strategies, and contribute to the aims of the EU Soil Strategy. The framework is grounded in scientific progress and adapted to fully support public and private land managers to take effective actions to protect, remediate and restore the environment on Europe's polluted soils.

ARAGORN will provide better insights into contaminated sites and improve remediation and restoration decision-making by implementing robust mapping and monitoring tools that are fit-for-purpose and covers a wide range of contaminants. Neglected hotspot polluted sites will be identified by fit-for-purpose monitoring and decision strategies. This will enable public and private stakeholders of contaminated sites to move from regrettable remediation to restorative remediation.

ARAGORN will compile and test remediation strategies and sustainable soil decontamination solutions, and will develop and put in practice nature-based solutions, improve knowledge on biodiversity and deliver a framework for step-by-step decision making in terms of what is the best approach for resilient restoration in various European countries. The complete framework will be developed together with strategic engagement of land managers throughout Europe, and by rooting knowledge through co-creative processes and sustainable infrastructures.

The implementation will be done through a strong team of multidisciplinary scientists and practitioners with ongoing commitments with a diverse set of stakeholders across Europe at the local, national and EU level. We will interlink land managers and sectors across Europe to take effective action on soil health and provide longevity links and support to several EU policy and international commitments.

### **6.03.P-We515 Using vis-NIR spectroscopy for the evaluation of environmental quality in agricultural areas potentially contaminated**

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The assessment of environmental quality of potentially contaminated areas intended for agri-food production pursuant to the Italian environmental law (DM 46/2019) provides for the adoption of specific criteria and investigation methodologies for the characterization of agricultural soils which, sometimes, require rather long and expensive acquisition and processing times.

As part of one of the first applications of DM 46/2019 to a large potentially contaminated area close to the city of Taranto (Southern Italy), the CNR-IRSA has experimented with an innovative investigation methodology, faster and cheaper than traditional analyses, which involves the use of visible-near infrared (vis-NIR) spectroscopy for the characterization of contaminated soils. This technology allows to quickly evaluate the nature and chemical, physical, and mineralogical properties of soils, and their state of contamination.

The spectral reflectance measurements, i.e. the ratio between the radiation reflected by a soil surface to that incident on it, were conducted in the laboratory on 108 composite soil samples deriving from the mixing of elementary samples taken in 73 homogeneous investigation areas, identified in the phase of characterization of the agricultural territories of Statte (TA) according to the methodology defined by the DM 46/2019. Particular attention was given to the analysis of PolyChlorinated Biphenyls (PCBs) which, in some composite samples, exceeded the regulatory limit (6 ng/kg), with maximum concentrations between 11.55 and 94.61 ng/kg. In fact, recent studies performed by the CNR-IRSA have demonstrated that high concentrations of PCBs can influence the color of the soil; therefore, in soils heavily contaminated by PCBs, it is possible to estimate with good accuracy the content of these contaminants through a simple determination of the color parameters by using a portable spectroradiometer; furthermore, by statistically relating spectral reflectance measurements to the results of laboratory chemical analyses, predictive models of some PCBs congeners can be calibrated by applying multivariate statistical methods.

Given its potential, vis-NIR spectroscopy can therefore represent a valid alternative to traditional analyzes for investigating potentially contaminated soils and be used both for pre-screening activities of the state of soil contamination and for the characterization and subsequent monitoring phases.

### **6.04 Environmental Toxicology and Chemistry in Africa: Exchanging Knowledge and Progress on Tackling Legacy and Emerging Pollutants**

#### **6.04.P-We516 Comprehensive Chemical Analysis to Unravel Exposure to Chemical Mixtures in Kenyan Rivers**

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Chemicals in the environment pose unprecedented challenges to human and aquatic health. The ubiquitous presence of these contaminants raises concerns about their cumulative impacts. Several studies have focused primarily on single pollutants, often overlooking the complex interplay of multiple mixtures coexisting in the environment. Technological advancement, modern analytical techniques have enabled a more comprehensive understanding of environmental exposures, revealing an array of extensive mixed-toxicant situations. This study employed a comprehensive chemical analysis to unravel the exposure of aquatic ecosystems to chemical mixtures. By using liquid chromatography and mass spectrometry, we identified and quantified a diverse range of chemical compounds present in water samples. In total, 307 compounds with concentrations ranging from 0.3 ng/L to 6.6 µg/L were identified, with at least forty-six (46) of them detected in more than 50% of the samples. Tetrabutylammonium and the pharmaceutical amantadine was detected in all the samples (100% detection) with concentrations ranging from 3 ng/L to 59 ng/L and 4 ng/L to 49 ng/L respectively. The wet season of October and the dry season of February recorded the highest percentage of chemicals exceeding 5 ng/L with respect to the detected number of compounds. Exposure risk for crustaceans was the highest with values of up to 60 times beyond the acute threshold level. The top contributors of toxicity included the pesticides diazinon, pirimiphos-methyl, clothianidin and imidacloprid. Acute threshold level for algae was exceeded in the dry season of July while significant TU values for fish was recorded during the wet season of May. It's crucial to recognize that the risks observed in aquatic organisms are indicative of potential risks on humans. Understanding these risks is paramount for safeguarding the environment in the broader context of chemical exposure. These findings provide valuable insights into chemical pollution in Kenya

#### **6.04.P-We517 Water Quality Challenges in South Africa: Gauging the Size of the Problem**

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Growing challenges of untreated wastewater effluent and runoff from agricultural landscapes are contributing to an approaching water quality tipping point in South Africa's rivers. However, not all rivers are equally vulnerable to this, or

impacted equally over the course of a hydrological year. Identifying high risk areas of water quality vulnerability, and likelihood of impact at different times of the year, requires an understanding of pollution loads relative to hydrograph and rainfall patterns. A simple modelling method was implemented to characterize river flow patterns into flushing or buffering states. Periods of vulnerability to point (typically as constant inputs throughout the year) and non-point (variable inputs driven by rainfall patterns) pollutants relative to flow volume and flooding helps to identify critical seasons when aquatic biota are most likely to be negatively impacted by poor water quality. Mean daily flow rate time series > 55 years duration were downloaded from the South African national hydrological services database for six gauging weirs occurring in three rainfall regions. Each flow data file was analysed with non-parametric statistics, using the Indicators of Hydrologic Alteration approach. Flow time series were divided into two equal parts, representing historical versus current periods. Metrics were calculated for each site that describes a hydrograph in terms of frequency, duration, timing and magnitude of hydrological events. Differences between catchment hydrology were characterised using descriptive and multivariate methods. We anticipate that this will enable rivers to be classified in terms of their relative flushing and buffering capacities as a function of their baseflow and predictability (flashiness) metrics. Linking this to a national flow type classification provides a spatial key to identifying which rivers are more likely to be resilient to water quality problems. This has applicability in screening of water use licenses and catchment management.

#### **6.04.P-We518 Let It Burn – Evaluation of Polycyclic Aromatic Hydrocarbon Emissions from Sugar Cane Pre-harvest Combustion**

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The pre-harvest combustion of sugar cane to remove unwanted biomass is common practice in a number of developing countries, including South Africa. Although guidelines are in place to protect farm workers and local communities from the vast amount of pollutants which are released to the atmosphere during sugar cane burns, these are typically limited to the maximum permitted wind speed and direction. This study sought to identify additional parameters to inform best practice and thereby reduce environmental pollution and human exposure to potentially toxic emissions arising from sugar cane burning. Polycyclic aromatic hydrocarbons (PAHs) were thus one of the important classes of semi-volatile organic compounds (SVOCs) which were investigated, in both the gas and particle-associated phases.

Monitoring was conducted during five disparate sugar cane burns at different locations in the KwaZulu Natal Province of South Africa. Simultaneous sampling of gas and particle bound SVOCs at each site before and after the burn event was achieved by means of portable multichannel polydimethylsiloxane denuders placed 16 m from the edge of the respective field. The denuders and associated quartz fibre filters were thermally desorbed with analysis by comprehensive two-dimensional gas chromatography coupled to a time of flight mass spectrometer. Total PAH concentrations (gas + particle phase) ranged from 0.05 to 9.85  $\mu\text{g m}^{-3}$  per individual burn event. Over 85% of all PAHs were found to exist in the gas phase, dominated by two- and three-ring PAHs. These gas phase analytes also caused the majority of variance between the burn sites. The potential carcinogenicity of the emissions based on toxicity equivalent quotients (TEQs) reflected the large variance in total PAH concentration per burn event, with the highest TEQ being 5.97  $\text{ng m}^{-3}$ .

Aside from the prevailing wind speed and direction, it was evident that the results in terms of PAH concentrations were also impacted by geographical location, as well as the sugar cane varietal which was being combusted. Additional laboratory based controlled burn studies conducted on the sugar cane waste material further illustrated the variable and complex mixture of the combustion derived SVOCs. The results of this study are important in informing best practice with respect to sugar cane burning for effective management and minimization of both environmental impacts, including climate change, and nuisance to the public.

#### **6.04.T-01 Learnings From the Environmental Pollution Programme in South Africa: Building a Legacy To Manage and Mitigate the Impacts of Pollution**

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Environmental pollution is one of the three interconnected planetary crises, along with climate change and biodiversity loss, which are placing global economic, environmental, and social well-being at risk. Pollution disproportionately affects the most vulnerable groups, with a higher proportion of annual pollution-related deaths occurring in low- to middle-income countries (LMICs). This motivated the UK's Department for Environment, Food and Rural Affairs (Defra) and the Joint Nature Conservation Committee (JNCC) to collaborate with partners across Africa and Asia on an Environmental Pollution Programme, funded by Official Development Assistance (ODA). The programme aims to build science-action partnerships for knowledge generation on managing and mitigating environmental pollution, through investments in human resources and action research. The presentation will share and reflect on the lessons learned in implementing ten research projects in South Africa through partnerships with local partners, from scoping phase to the development of a multi-year programme tackling environmental and regulatory challenges. Interventions in the form of pilot-scale solutions to mitigate pollution range from, the development of a water quality costing model, community-led management of solid waste and springs, to developing

biological solutions to treating dairy wastewater. The learnings gained to date justify the replicability of the programme, particularly in other LMICs to share knowledge and ensure the legacy of the programme. This presentation will be used to reflect on these learnings in the hope of creating synergies with similar efforts globally.

#### **6.04.T-02 Tackling Diaper Pollution: Enhancing Soil Moisture, Biomass Accumulation, and Growth Patterns of Napier Fodder through Entrenched Diapers and Biochar in a Degraded South African Landscape**

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Many South African communities lack waste collection services. While diapers are convenient and affordable for modern households, their utilization is negated by indiscriminate disposal, including landfilling, which threatens the environment. Additionally, areas in the Upper uMkhomazi Catchment, South Africa endure extensive land degradation and *Acacia mearnsii* encroachment which are global concerns. Communal farmers in the area also experience shortages in livestock fodder, particularly in winter. This study seeks to tackle this multifaceted challenge and explore the impact of entrenching diapers with biochar from invasive *A. mearnsii* on soil moisture, growth, and dry matter (DM) of Napier fodder.

A field experiment was conducted on degraded lands in South Africa. The treatments were control (CON), nappies only (NO), biochar only (BO), nappies + biochar (NB), nappies + fertilizer (NF), fertilizer + biochar (FB), and nappies + fertilizer + biochar (NFB) treatments. The nappies, biochar and fertilizer were entrenched, and Napier fodder was planted. Soil moisture was recorded by a H2S CS658 probe. Tiller numbers, plant heights and leaf chlorophyll content (SPAD-based) were also monitored, and the plants were harvested for DM yield 20 weeks after planting. Overtime towards winter, NFB, NF, NO, and NB showed a significant decline in soil moisture compared to CON, BO, and FB. The tiller numbers ranged from 3 (BO) to 14 (NF) tillers per plant. Plant heights increased with increasing growth period, with NF, NFB, and FB significantly taller followed by NB and NO, and lastly CON and BO. The NFB and NF treatments had significantly greater ( $p < 0.001$ ) leaf chlorophyll compared to the CON. DM yield ranged from 0.217 to 2.13 ton/ha, with NFB and NF having significantly the highest, while BO had the lowest. Monitoring for microplastics also occurred.

The findings of this study suggest that entrenching nappies and biochar addresses challenges of disposal of nappies, black wattle invasion, and fodder shortages through increased productivity of degraded lands.

#### **6.04.T-03 Sustainable Waste Management in Ghana: An In-depth Analysis of the Dompouse Landfill Site and Its Implications for Community Development and Perceived Health risk**

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In numerous African countries, inadequate solid waste management systems have led to heightened health risks for communities residing near landfill sites. This study aims to enhance our understanding of the health implications associated with living near the Dompouse landfill site in Kumasi, Ghana. Through an integrated approach involving GIS mapping, proximity analysis, and questionnaire surveys, the research investigates the existing landfill boundaries, assesses proximity-related health risks, and identifies key factors influencing public perception. A total of 400 questionnaires were randomly distributed among residents in Kuwait, Adagya, Aprabon, and Sokoban, areas surrounding the Dompouse landfill. Chi-square analysis was employed to scrutinize associations among variables. Utilizing the ring buffering analysis in ArcGIS, the study determined households and institutions in proximity to the landfill. Remarkably, 92.8% of respondents acknowledged a perceived health risk linked to living near the landfill. Categorizing the perceived health risk, 14.5% reported very high exposure, 34.8% high exposure, 21.8% moderate exposure, 20.8% slight exposure, and 8.3% deemed no risk. Significant influencers on perceived health risks included age, duration of residence, and the environmental and social impacts of the landfill.

#### **6.04.T-04 The utility of environmental monitoring in understanding the recovery of estuarine habitats subjected to chronic and acute stressors: the case of the uMhlanga estuary in South Africa**

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Environmental monitoring is critical in facilitating timely and prioritised decision-making for habitat restoration. This study focuses on the uMhlanga River and Estuary in South Africa, which has been subjected to long term chronic anthropogenic stressors and a catastrophic agrochemical spill in July 2021.

In response, remediation, rehabilitation, and monitoring programs have been initiated, and evidence of system recovery has been documented. We seek to answer the question: what are the most effective monitoring tools and approaches, for providing the necessary information to inform effective decision making for restoration and rehabilitation of polluted estuarine environments. This study assesses the utility a range of monitoring tools to track the recovery of the uMhlanga estuarine system, comparing it with reference sites (Mpenjati and uMdloti estuaries) to identify appropriate indicators for to understand recovery trajectories towards a reference condition.

The research employs a comprehensive approach, considering water quality indicators, remote sensing for vegetation mapping, blue carbon stocks, fish assemblages, bioindicators, and historical data. In this presentation, we focus on bioindicators and water quality indices, presenting findings for the uMhlanga and reference estuary sites.

Results indicate a significant impact on water quality parameters in the uMhlanga River and Estuary. Diatom data reveal elevated toxin concentrations in May 2022, evidenced by valve deformities exceeding threshold limits which were absent in a subsequent assessment in August 2023, suggesting system recovery. However, the prolific presence metal-tolerant diatoms indicate the potential presence of residual toxins.

Our initial results suggest that there is utility in the approach, yet more data is needed to confirm most effective indicators for tracking estuarine ecological recovery post-pollution events. This research contributes valuable insights into the recovery dynamics of estuarine ecosystems, essential for informed decision-making and effective interventions.

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#### **6.04.P Environmental Toxicology and Chemistry in Africa: Exchanging Knowledge and Progress on Tackling Legacy and Emerging Pollutants**

##### **6.04.P-We516 Comprehensive Chemical Analysis to Unravel Exposure to Chemical Mixtures in Kenyan Rivers**

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Chemicals in the environment pose unprecedented challenges to human and aquatic health. The ubiquitous presence of these contaminants raises concerns about their cumulative impacts. Several studies have focused primarily on single pollutants, often overlooking the complex interplay of multiple mixtures coexisting in the environment. Technological advancement, modern analytical techniques have enabled a more comprehensive understanding of environmental exposures, revealing an array of extensive mixed-toxicant situations. This study employed a comprehensive chemical analysis to unravel the exposure of aquatic ecosystems to chemical mixtures. By using liquid chromatography and mass spectrometry, we identified and quantified a diverse range of chemical compounds present in water samples. In total, 307 compounds with concentrations ranging from 0.3 ng/L to 6.6 µg/L were identified, with at least forty-six (46) of them detected in more than 50% of the samples.

Tetrabutylammonium and the pharmaceutical amantadine was detected in all the samples (100% detection) with concentrations ranging from 3 ng/L to 59 ng/L and 4 ng/L to 49 ng/L respectively. The wet season of October and the dry season of February recorded the highest percentage of chemicals exceeding 5 ng/L with respect to the detected number of compounds. Exposure risk for crustaceans was the highest with values of up to 60 times beyond the acute threshold level. The top contributors of toxicity included the pesticides diazinon, pirimiphos-methyl, clothianidin and imidacloprid. Acute threshold level for algae was exceeded in the dry season of July while significant TU values for fish was recorded during the wet season of May. It's crucial to recognize that the risks observed in aquatic organisms are indicative of potential risks on humans. Understanding these risks is paramount for safeguarding the environment in the broader context of chemical exposure. These findings provide valuable insights into chemical pollution in Kenya

##### **6.04.P-We517 Water Quality Challenges in South Africa: Gauging the Size of the Problem**

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Growing challenges of untreated wastewater effluent and runoff from agricultural landscapes are contributing to an approaching water quality tipping point in South Africa's rivers. However, not all rivers are equally vulnerable to this, or impacted equally over the course of a hydrological year. Identifying high risk areas of water quality vulnerability, and likelihood of impact at different times of the year, requires an understanding of pollution loads relative to hydrograph and rainfall patterns. A simple modelling method was implemented to characterize river flow patterns into flushing or buffering states. Periods of vulnerability to point (typically as constant inputs throughout the year) and non-point (variable inputs driven by rainfall patterns) pollutants relative to flow volume and flooding helps to identify critical seasons when aquatic biota are most likely to be negatively impacted by poor water quality. Mean daily flow rate time series > 55 years duration were downloaded from the South African national hydrological services database for six gauging weirs occurring in three rainfall regions. Each flow data file was analysed with non-parametric statistics, using the Indicators of Hydrologic Alteration approach. Flow time series were divided into two equal parts, representing historical versus current periods. Metrics were calculated for each site that describes a hydrograph in terms of frequency, duration, timing and magnitude of hydrological events. Differences between catchment hydrology were characterised using descriptive and multivariate methods. We anticipate that this will enable rivers to be classified in terms of their relative flushing and buffering capacities as a function of their baseflow and predictability (flashiness) metrics. Linking this to a national flow type classification provides a spatial key to identifying which rivers are more likely to be resilient to water quality problems. This has applicability in screening of water use licenses and catchment management.



#### **6.04.P-We518 Let It Burn – Evaluation of Polycyclic Aromatic Hydrocarbon Emissions from Sugar Cane Pre-harvest Combustion**

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The pre-harvest combustion of sugar cane to remove unwanted biomass is common practice in a number of developing countries, including South Africa. Although guidelines are in place to protect farm workers and local communities from the vast amount of pollutants which are released to the atmosphere during sugar cane burns, these are typically limited to the maximum permitted wind speed and direction. This study sought to identify additional parameters to inform best practice and thereby reduce environmental pollution and human exposure to potentially toxic emissions arising from sugar cane burning. Polycyclic aromatic hydrocarbons (PAHs) were thus one of the important classes of semi-volatile organic compounds (SVOCs) which were investigated, in both the gas and particle-associated phases.

Monitoring was conducted during five disparate sugar cane burns at different locations in the KwaZulu Natal Province of South Africa. Simultaneous sampling of gas and particle bound SVOCs at each site before and after the burn event was achieved by means of portable multichannel polydimethylsiloxane denuders placed 16 m from the edge of the respective field. The denuders and associated quartz fibre filters were thermally desorbed with analysis by comprehensive two-dimensional gas chromatography coupled to a time of flight mass spectrometer. Total PAH concentrations (gas + particle phase) ranged from 0.05 to 9.85  $\mu\text{g m}^{-3}$  per individual burn event. Over 85% of all PAHs were found to exist in the gas phase, dominated by two- and three-ring PAHs. These gas phase analytes also caused the majority of variance between the burn sites. The potential carcinogenicity of the emissions based on toxicity equivalent quotients (TEQs) reflected the large variance in total PAH concentration per burn event, with the highest TEQ being 5.97  $\text{ng m}^{-3}$ .

Aside from the prevailing wind speed and direction, it was evident that the results in terms of PAH concentrations were also impacted by geographical location, as well as the sugar cane varietal which was being combusted. Additional laboratory based controlled burn studies conducted on the sugar cane waste material further illustrated the variable and complex mixture of the combustion derived SVOCs. The results of this study are important in informing best practice with respect to sugar cane burning for effective management and minimization of both environmental impacts, including climate change, and nuisance to the public.

#### **6.04.P-We519 Introduction to the Session on Understanding Pollution Issues Facing Low- And Middle-Income Countries, Sharing Knowledge with the Global South**

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This series of presentation attempts to highlight the disparity between the global North and South in recognising and responding to the impacts of pollution on reducing biodiversity; often exacerbated by climate change and key threats to global human health. In terms of pollution reduction, international donor countries representing the global north have failed to respond to this growing public health crisis. Given the severity of pollution on the public health burden, there is a critical need for aid funding to be allocated specifically to pollution reduction. Data from 2016, have shown an average investment of \$14/death for pollution, compared with for example, \$1,250/ death for malaria and \$190/ death for tuberculosis. People living in the world's poorest nations have many competing priorities, but such should not be used as an excuse to ignore environmental pollution within Official Development Assistance (ODA). What is required is recognition of these conjoined threats, a globally supported, formal science-policy interface to inform intervention, influence and share research and focus on policy developments. Viewed historically as a local issue to be addressed through local and national legislation, or through adopting policy from higher-income countries; it is now, however, increasingly obvious that with increasing industrialization and social-economic development, human activities have generated a series of global environmental issues, which in turn have brought health hazards and disease burdens to both the environment and to human society. Furthermore, solutions are linked to the complex relationship between environmental harm and economic development which sits within a broader pollution-economic nexus. As we consider a societal shift towards a circular economy, there is a need to consider a more shared and integrated frameworks for analysing the empirical evidence that connects pollution and development, and its implications for human well-being and the achievement of the sustainable development goals in the global south. The session is intended to share outcomes of pilot scale solutions to improve environmental quality and approaches to control or mitigate the consequences of pollution and build future opportunities for better regulatory controls. Presentations have focussed on solutions to address the perspective from the Global South and consider the barriers and regulatory restrictions that hinder knowledge sharing.

#### **6.04.P-We520 Metal Element and Organochlorine Pesticide Levels in Elasmobranchs from the East and South Coast of South Africa.**

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In 2011, a total of 81 elasmobranch species were classified as threatened within South African waters. The continuous increase of pollutants, caused by anthropogenic pressures, is a major contributor to the stress experienced placed on these species. As South Africa is mineral resource rich country, elements enter the marine environment through terrestrial run-off. In addition, many organochlorine pesticides that have been banned are still applied and therefore also end up in the marine environment. The aim of this study was to determine levels of selected elements and organochlorine pesticides (OCP) in 21 shark, ray and skate species along the South African coast. Muscle samples were prepared for element and OCP analyses using standard acid digestion and organic solvent extraction and clean-up procedures. Elements were analysed using ICP-MS and GF-AAS; and OCPs using GC-MS techniques. Clear spatial differences along the coastline as well as differences in element accumulation patterns between different types of elasmobranchs were observed. Benthic feeding species e.g., batoids, accumulated higher concentrations of Fe, Zn, and As and the shyshark species accumulated the highest levels of potentially toxic elements, i.e. Cd and Pb. The element accumulation fingerprint revealed regional specific exposure patterns. Of the 22 OCPs analysed only nine compounds were detected. The DDT and its isomers occurred in the highest concentrations, followed by HCBs and chlordanes. Accumulation patterns were related to intrinsic factors (e.g. sex and trophic position) as well as external factors such as migration and foraging behaviour. Shark species (e.g. White sharks, spinner and dusky sharks) that follow transoceanic migration patterns had high DDT levels which is the result of exposure from multiple sources and regions. However, high OCP concentrations were also recorded in philopatric species such as the scalloped hammerhead and bull shark, which have more restricted movements in and around nearshore and shallower regions. Thus, the exposure fingerprints were indicative of more regional OCP exposure patterns. There is currently no tissue residue-related measure of elasmobranch health. However, based on residue levels that are deemed safe for human consumption, most of the species could be regarded to be in a healthy condition.

#### **6.04.P-We521 Integrated Knowledge Systems Towards Flood Resilience and Sustainable Solid Waste Management in South African Urban Informal Settlements**

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Climate change, an increasing urban population, and poor urban planning have increased flood-risk and the accompanying solid waste challenge in many coastal urban areas in developing countries. These challenges are more pronounced in informal settlements because: (a) they are often built on environmentally fragile locations such as river banks and coastal shores with high exposure to floods, (b) high poverty levels among residents resulting in low adaptive capacity, and (c) marginalisation of these localities emanating from their non-recognition in the larger city framework. Against this background, flood-risk assessments and response initiatives in these areas have primarily been informed by scientific approaches such as geographical information systems, without adequate incorporation of other forms of knowledge. Using the case of the coastal city of Durban, South Africa, our project explores the benefits of combining perspectives from different knowledge systems in understanding flood-risk and the accompanying solid waste challenge in urban informal settlements, towards developing solutions that are based on contextual and experiential aspects. Methodological techniques used include interviews and workshops with key experts and with informal settlement residents, and extensive reviews of literature. Emerging findings show that holders of scientific, practitioner, and local knowledge vis-à-vis flood risk and waste management are active in the selected case study informal settlement. They have, in isolated cases, collaborated particularly around a) generation and distribution of flood early warnings, b) river clean-up initiatives, and c) catchment rehabilitation projects, with clear benefits for flood resilience and solid waste management. We find that there is need for a clear framework for integrating knowledge systems towards flood resilience and solid waste management in these contexts and the project has developed a draft framework. Integrating knowledge systems will: i) ensure the participation of different actors in mapping flood risk thereby creating a sense of ownership and ensuring uptake of and support for solutions crafted to deal with flood risk and the solid waste challenge; and ii) open up opportunities for coordinated support from various actors for a range of decisions around flood risk response preparation, flood and waste infrastructural design and mitigation of waste-induced flood destruction of infrastructure.

#### **6.04.P-We522 Achieving Community-Led Solid Waste Pollution Mitigation at the Catchment Scale: The Case of the Umkhomazi and Umngeni Catchments in Kwazulu-Natal, South Africa**

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Solid waste management (SWM) is a pressing and complex global problem demanding continuous efforts. SWM is not receiving the required attention compared with other sectors, and over half of solid waste is openly dumped, particularly in developing countries. In South Africa, pollution is closely linked with ineffective management of solid waste. Several municipalities in the country are battling to deliver effective SWM. Budget constraints are the main hindrances to proper management of solid waste, with the lack of awareness contributing to the escalation of pollution. This project aims to work with the communities to co-develop proper disposal and possible repurposing of solid waste. The project applied a mixed-method approach using desktop research, workshops and surveys. The project sites range from rural (Amangwane), peri-urban (Impendle) and urban (Sobantu) areas. Waste audit was conducted to identify waste types on illegal dumping hotspots within each area. Research findings will be used to inform awareness campaigns which will be implemented in each area. Consistent trends of certain waste types were observed across the three areas. All three study areas possessed similar waste disposal habits i.e. dumping waste on vacant fields within overgrown vegetation, nearby streams and rivers. Lack of waste collection services

and inadequate knowledge of proper waste disposal seemed to be the main causes of illegal dumping. The need to conduct an awareness campaign on solid waste pollution came out strongly from all three communities. There is a significant difference in waste generated between each community, with Sobantu leading followed by Amangwane and lastly Impendle. Plastic waste was generally high across all three communities, with Sobantu having the highest accumulation followed by Amangwane and Impendle. Nappies were also recorded to be high in Amangwane during the waste audit as was indicated during the workshops but in Impendle and Sobantu, the nappies were relatively lower compared to what was indicated during workshops in these communities. The inconsistencies within the study with regard to waste quantities in urban, peri-urban, and rural compared to what is reported in the literature require further investigation. The waste audit provided insights into the most problematic waste types and this information will assist with determining the required interventions.

#### **6.04.P-We523 Human Health Risk Assessments of Selected Metals in the Qwaqwa Region River Waters**

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Metal pollution is a global concern owing to its toxic and bioaccumulative properties and tendency to persist in the environment. Pollution of aquatic ecosystems has attracted considerable attention globally; however, not much attention has been given to waterheads, specifically in the QwaQwa region. We selected four major streams in the Qwaqwa region (Namahadi, Metsi-Matsho, Mphukojwane and Elands Rivers) that are important to the upper Vall River basin. We aimed to assess the water quality across the selected rivers, compare it with the national guidelines, and use indices to estimate health risks. We mainly focused on the variations between the upper, middle, and densely populated lower reaches. The selected sites were sampled over two years during dry and wet seasons. The physicochemical parameters and concentrations of 10 elements (As, Al, Ca, Cu, Fe, Mg, Mn, Sr, V) were determined in water samples. Our findings revealed that concentrations ranged from below the detection limit to an average of 15.23 mg/L in water samples, where Ca, Mg and Mn were the main contributors to the total concentrations. Compared with Target Water Quality Ranges for Aquatic Ecosystems, Al, Cu, Mn, and Zn concentrations were above permissible limits in all sample sites. On the other hand, Al, Fe, and Mn concentrations revealed a general exceedance of Target Water Quality Ranges for Domestic Use standards. Regarding the total concentration, the Mphukojwane River's upper and middle sites were the most polluted, followed by the Elands River, the lowermost site. Average Daily Dose (ADD) estimates for Mn in the MPU site and Sr in the MPM & EL sites water were higher than the acceptable reference dose for the ingestion route. A significantly higher ADD value for Cu was observed in most sites except for EL and MM. Moreover, Mn at Mphukojwane upper, Sr at Elands and Mphukojwane middle had a Hazard Quotient value higher than the threshold value of one for dermal exposure routes. Most rivers displayed an increase in the rate of pollution from the upper to the lower reaches, which highlights the pressure of industrialization and other factors. These findings are of specific significance for the utilisation and protection of water resources in the basin and the protection of public health risks emanating from poor water quality.

#### **6.04.P-We524 Microplastics across the drinking water supply chain of Addis Ababa, Ethiopia.**

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Owing to their excellent versatility, durability, relatively low production costs and inherent advantages that promote diverse applications, plastics provide enormous societal benefits ever since its mass production started in the 1950s. Nonetheless, due to aging and fragmentation processes of the residue of plastic wastes, microplastics are formed and become a pressing global concern as they are closely tied to human health.

Different studies have proven that microplastics are almost everywhere including the air we breathe, the food we eat, and the water we drink, with a diverse range of polymer types, shapes, and sizes. However, there is little information about the extent of microplastics in drinking water systems of middle and low income countries, like Ethiopia, where pervasive plastic wastes are serious threats. The situation gets even worse in highly populated areas, where efficient, economical and technologically sound drinking water treatment plants, that provide a barrier for microplastics accessing the drinking water, are lacking.

Addis Ababa, the capital of Ethiopia, is the primary city with more than 5 million inhabitants and in which more than 70% of them are living in slum areas. Despite this large number of people, very little attention is given for drinking water supply infrastructures including advanced drinking water treatment plants.

In this research, the relative distribution of microplastics across the drinking water production line in Addis Ababa was determined. Accordingly, water samples were collected from raw water and treated water outlet of the largest conventional drinking water treatment plant in Addis Ababa, from storage tank and from municipal drinking water taps both in standard and slum areas of the city both during dry and wet seasons. Microplastics were extracted and identified using Fourier transform infrared spectroscopy.

This study is conducted because of the need in bridging the knowledge gap concerning microplastics distribution in drinking water from low and middle income countries. In addition, the efficiency of the available conventional drinking water treatment

plant in removing microplastics and the contribution of the piping system across the drinking water distribution network for microplastics in the tap water will be determined. This research can support the development of appropriate mitigating measures and standards for better plastic management.

Keywords: Microplastics, drinking water, Ethiopia

#### **6.04.P-We525 Spring Protection For Sustainable Water Supply: A Case Study Of Water Use And Quality Within Two Selected Sub-Catchments In Kwazulu-Natal Province, South Africa**

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In South Africa many rural areas lack access to formal water services and about 80% of rural villages rely on springs to satisfy their water needs. Unfortunately, the physical, chemical and biological quality of the spring water consumed by communities is often compromised by a combination of factors such as livestock trampling, contaminated surface runoff, pollution, and poor management of the broader area. Consumption of microbial-contaminated water has severe implications for human health. Globally, over 50 kinds of diseases are caused by poor quality drinking water quality, and 80% of diseases and 50% of child deaths are related to poor drinking water quality. The project aimed to provide a comprehensive analysis of water quality of natural springs and co-develop interventions that protect the springs and enhance ecological infrastructure through local landscape restoration and rehabilitation. Surveys were conducted in 77 households in four rural communities (i.e. Mbhava, eMambedwini, Ndonyela and Sambane) of KwaZulu-Natal province to determine their water-use practices, perceptions of water quality, and household water-treatment methods. Drinking water samples from abstraction and collection points were tested for physicochemical and microbiological quality in the dry and wet seasons. All participants used spring water for drinking, cooking, washing and cleaning. Except for eMambedwini spring users, over 96% of participants perceived that springs were facing pollution challenges from contaminated surface runoff, human and livestock interference. Over 73% of participants did not treat spring water before use. Water from three springs tested positive for *Escherichia coli* and total coliforms and in some cases high levels of nitrate. The general trend was that densities of *Escherichia coli* and total coliforms were lower in the dry season due to lower discharge of contaminated surface runoff into the spring sources. The findings of this study highlighted that rural communities in KwaZulu-Natal rely on poor quality spring water to meet their household water needs. Furthermore, the presence of high levels of *E. coli* and total coliforms found in spring water has severe implications for human health, and authorities should strengthen spring protection interventions to minimise pollution and improve water quality. There is also a need to create awareness about potential hazards associated with drinking water from natural sources.

#### **6.04.P-We526 Application of bioassays for the evaluation of aquatic toxicity of a WWTP effluent in Western Cape, South Africa**

**Sihle Mlonyeni, Omoniyi Kolawole Pereao and Beatrice Olutoyin Opeolu, Cape Peninsula University of Technology, South Africa**

Management of wastewater from diverse sources is important because effluents are discharged into the aquatic environment after treatment. In this study, the water quality of the Veldwachters River and associated ecotoxicity risks of the wastewater treatment plant's (WWTP) effluent were evaluated. Physicochemical characterisation over four seasons and ecological risk assessment of WWTP's effluent using aquatic organisms comprising the producer *Raphidocelis subcapitata*, consumer *Daphnia magna* and decomposer *Tetrahymena thermophila* as experimental models were investigated. The crustaceans were subjected to 48 h exposure for mortality while algae and the protozoan were exposed for growth inhibitions at 72 h and 24 h, respectively. Physicochemical characteristics were within regulatory limits. Growth inhibition was observed in autumn and winter for *R. subcapitata* and in summer and spring for *T. thermophila*. Crustaceans *D. magna* (consumer) was classified as Class III (acute toxicity) for the effluent in all seasons. Meanwhile, the most sensitive organism in the applied battery of biotests was the protozoan *T. thermophila* (decomposer), demonstrating a toxic unit (TU) > 100, while *R. subcapitata* and *D. magna* demonstrated 1 TU < 10. These results showed that the effluent has potential toxicological effects on aquatic organisms and provided insights into the required intervention strategies for pollution reduction.

#### **6.04.P-We527 Examining Polycyclic Aromatic Hydrocarbons in the Orange-Senqu River Catchment: Impact on Ecosystems and Health**

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The transnational expanse of the Orange-Senqu River Catchment (OSRC), spanning Botswana, Namibia, Lesotho, and South Africa (SA), faces a significant ecological challenge marked by the pervasive presence of polycyclic aromatic hydrocarbons (PAHs). This study scrutinises the specific concern of PAHs, identified by the United States Environmental Protection Agency (USEPA) as sixteen priority congeners necessitating monitoring and regulation due to their deleterious effects on wildlife and human health. This study delves into the specific concern of PAHs, employing a comprehensive analysis of sediment samples collected from 75 sites. Multiple toxicity assessment approaches were employed: (1) toxicity, quality, and ecological risk were evaluated by comparing PAH concentrations to an international sediment quality guideline (SQG); (2) the sediment quality guideline index (SQG-I) was calculated; (3) risk quotients were determined; and (4) carcinogenic and mutagenic potentials were explored in terms of benzo(a)pyrene-equivalent assessments, quantifying total benzo(a)pyrene (B[a]P) equivalent concentrations ( $\sum_{16}B[a]P_{TEQ}$ ) and mutagenic equivalents ( $\sum B[a]P_{MEQ}$ ). Individual PAH concentrations exhibited a range of

0.5–1001 ng/g dry mass (dm), with cumulative PAH levels in sediment (2.4–4969 ng/g dm). The eastern region of the OSRC displayed moderate to high PAH contamination. The SQG and SQG-I pinpointed specific sites in SA with anticipated toxicity. The highest  $\sum_{16}B[a]P_{TEQ}$  value was 530 ng/g dm, with substantial contributions from B[a]P, benzo[b+k]fluoranthene, and dibenzo[ah]anthracene. Despite localised hotspots, low carcinogenicity was suggested for the majority of the OSRC. However, three sites exhibited high carcinogenicity and mutagenicity ( $\sum B[a]P_{MEQ}$  555–634 ng/g dm). Projections suggest a risk of moderate to high toxicity and suboptimal sediment quality for benthic organisms, highlighting concerns about potential adverse ecological impacts and health risks. Elevated contamination at specific sites in SA was attributed to industrialisation, emphasising the urgent need for targeted interventions to mitigate PAH-related risks in the OSRC. This study provides crucial insights into the complex interplay between PAHs and the aquatic environment. It informs future regulatory frameworks, underscoring the significance of proactive measures to protect the well-being of ecosystems and human populations.

#### **6.04.P-We528 Abidjan Convention and the Chemical Management Strategy**

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Protection of the marine environment from the discharge of chemicals used offshore is of global importance. Sustainable operations in the oil and gas sector requires a regulatory framework ensuring protection of the ecosystem and prevention of deleterious environmental impact.

The Convention on Cooperation for the Protection, Management and Development of the Marine Environment and Coastal Areas of the West, Central and South Africa Region (Abidjan Convention) has been instigated with the rationale of integrating a concerted management of regional ecosystems. Pertaining to the Convention, the Malabo Protocol provides Contracting Parties of the region with a common framework to better supervise the development of these activities and ensure their compatibility with the protection of the marine environment. This development came in response to the accelerated development of offshore oil and gas activities in the region. The implementation plan fundamentally requires a clear understanding of a viable strategy, taking into account the elements and aspects that currently exist.

The objective of this conveyance is to examine the pertinent considerations of adaptation of chemical management strategy from a global perspective. The goals and plan to align with existing international standards will be assessed. Implications of capacity and infrastructure to achieve defined standards and the need for scientific and technical co-operation to facilitate progress will be analysed. Challenges and concerns focused on categorisation of chemicals and ecotoxicological testing will be addressed, as well as monitoring programmes and implementation procedures and overall objectives.

In essence the existing challenges of the Malabo Protocol will be highlighted, and potential scope for prudent course of action to advance the mission of the Abidjan Convention will be portrayed. This fulfils the aim to “Protect, Conserve and Develop the Abidjan Convention Area and its Resources for the Benefit and Well-being of its People.”

#### **6.04.P-We529 Investigating the feasibility of phycoremediation for dairy milking parlour wastewater in South Africa.**

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The dairy industry in South Africa is important for the local economy and yet, is a significant contributor to the countries wastewater pollution burden. We share the results of phycoremediation experiments involving the use of microalgal consortia to treat dairy wastewater (DWW). This is relevant given that South Africa is a lower-middle income country, with limited information available to help farmers to efficiently treat their wastewater while recovering essential nutrients, particularly in light of diminishing freshwater reserves and rising fertiliser costs.

Four dairy farms in KwaZulu-Natal Province, participated in the research where detailed wastewater audits were conducted and the physico-chemical constituents of the wastewater determined. Monthly samples were used for characterisation of the wastewater. Subsamples were treated with algal consortia inoculum and incubated to enrich the microalgae consortia. The enriched consortia were used for further phycoremediation experiments. Comparative growth profiles were analysed employing different concentrations of wastewater and the biodegradation kinetics were assessed to evaluate nutrient removal potential.

The observation of algal consortium growth in the dilutions indicates a positive response to certain concentrations of DWW. However, a notable growth limitation was noted as DWW concentrations increased. This limitation is attributed to the elevated turbidity, which impedes light penetration, hindering the essential photosynthetic activity of algae. The findings from the biodegradation kinetics revealed promising potential for nutrient removal in the treatment of dairy wastewater. However, the observed efficacy suggests the necessity for further adaptation experiments to augment removal efficiency. Future research aims to formulate strategies that enhance the removal of chemical oxygen demand. Additionally, the results spark curiosity about the possible involvement of native microorganisms present in dairy wastewater slurry ponds. These microorganisms may have adapted to the unique constituents of the wastewater, prompting the need for in-depth exploration. Investigating the role of these indigenous microorganisms holds promise for refining wastewater treatment processes and underscores the importance of ongoing research in this domain.

#### **6.04.P-We530 Odonata as indicators of heavy metals and environmental impact on an operational gold mine in South Africa.**

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Odonata occupy a dual niche perspective of both prey and predator in terrestrial and aquatic environments. Adult Odonata can be located near most freshwaters during summer, even those on an operational gold mine.

The mining industry in South Africa contributed over 12 billion US dollars to the South African GDP for 2021. The number of illegal miners is also increasing. Mining regulations require regular investigation into the impact of operations on the environment. These investigations will ultimately aid in decision-making in rehabilitation and mine closure efforts. Biotic and abiotic samples were collected.

Adult and nymph Odonata and sediment were collected over two years from seven aquatic locations in an operational gold mine in South Africa. Sampling locations included mine catchment dams, drainage ditches, return water dams, tailings storage facilities, and natural watercourses. Three additional locations served as reference locations. Additionally, species surveys were conducted for adult dragonflies to award each location with a dragonfly biotic index score (DBI) using the Dragonfly Biotic Index Manual of Freshwater Assessment for South Africa.

All samples were analysed for 28 metallic elements using ICP-MS at an accredited laboratory. Adult Odonata were located and sampled at all sample locations. However, nymphs were not found at three sites, while two additional locations had very few. Sediment samples collected from these sites had arsenic, cadmium, chromium, copper, lead, mercury, and zinc concentrations exceeding the probable effect levels of the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life. Heavy metal concentrations in adult dragonflies were generally lower than concentrations quantified in nymphs from the same locations. The analytical results and DBI scores aided in identifying heavy metal hotspots and would aid in future rehabilitation efforts.

#### **6.04.P-We531 Occurrence, Elimination and Risk Assessment of Organic Micropollutants: A Case Study of Selected Wastewater Treatment Plants in Western Kenya**

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Inefficient wastewater treatment plants (WWTPs) in addition to poor waste disposal strategies and sanitation facilities has resulted to pollution of surface and ground water systems. Occurrence of these compounds in the aquatic environment pose adverse effects to exposed organisms. Despite continuous consumption of these chemicals, monitoring has been inadequate especially in developing countries. This study aimed at identifying OMPs and their transformation products (TPs) in selected WWTPs as a route to human exposure, evaluating the removal efficiency, and performing risk assessment of OMPs present in the effluent on standard test organisms. Four WWTPs were selected and 400 mL of water sampled from the influent and effluent. Solid phase extraction was performed and analysis done using Liquid Chromatography coupled to a High-Performance Mass Spectrometer. Toxic unit (TU) approach was applied for risk assessment. A total of 310 compounds were detected across the WWTPs comprising of pharmaceuticals, pesticides, TPs and industrial compounds. Pharmaceuticals had the highest compounds detected (90). Compound concentrations were up to 841 µg/L (caffeine), 43 µg/L (2-oxindole), 24 µg/L (ibuprofen) in the influent and 830 µg/L (caffeine), and 95 µg/L (lauramidopropylbetaine) in the effluent. TPs were detected at high concentrations including deoxycholic acid (719 µg/L). Compounds such as cholic acid, 2,4-Dichlorophenol, and propylparaben were removed efficiently (100%) whereas others such as carbamazepine (-93%) and acetamiprid (-74%). From the risk assessment, crustaceans showed the greatest potential risk of toxicity with diazinon, bendocarb and carbendazim driving this risk. Low but substantial risk was observed for fish and algae with TU up to 0.5. Based on frequency and extent of exceedance of acute and chronic thresholds, 16 compounds including diazinon, and carbendazim by were prioritized for monitoring and abatement in western Kenya. This study shows the presence of OMPs in WWTPs and the potential risk to aquatic organisms. Invertebrates were shown to have greatest potential risk for toxicity compared to algae and fish with selected compounds driving the toxicity. These risk driving compounds have been prioritized as candidate compounds for monitoring and abatement in Kenya.

#### **6.05.P Flame Retardants and Regulation, Connecting Substance Grouping and Circular Economy**

##### **6.05.P-Mo501 Implications of lowering Low POP Content Limit values for the Circular Economy**

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To minimise unintentional contamination of articles with restricted brominated flame retardants (BFRs) because of recycling plastic items treated with BFRs, the European Union has introduced Low POP Content Limit (LPCL) values for waste, that if exceeded mean that the articles in question cannot be recycled. LPCLs were initially set at 1,000 mg/kg, but are set to be

lowered progressively to 500, 200, and 100 mg/kg in the next few years. Examination of data generated by a comprehensive survey of BFRs in waste articles in Ireland in 2019–20 identified that approximately 2,800 t/yr exceeded the LPCL value in force at that time. Enforcement of this 1,000 mg/kg limit for polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD), and tetrabromobisphenol-A (TBBP-A) would remove 78% of these BFRs from the Irish recycling stream. This demonstrates the effectiveness of the LPCL approach to minimising recycling of BFRs. Reducing LPCL concentrations would increase the proportion prevented from being recycled to 82, 84, and 85% depending on whether the limit was 500, 200, or 100 mg/kg respectively. Set against this, while enforcement of a 1,000 mg/kg LPCL would render 3.1% of the mass of the waste categories examined unrecyclable; this increases to 4.0, 4.9, and 5.6% when the LPCL is 500, 200, and 100 mg/kg respectively. Further research into the implications of such changes to limit values is recommended.

#### **6.05.P-Mo502 Illegal Recycling is not Circularization: Extremely High Levels of Legacy Brominated Flame Retardants in Children's Toys from European Markets**

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Given that 90% of toys on the market contain plastics, there are concerns about the presence of hazardous chemicals like polybrominated diphenyl ethers (PBDEs) due to the use of recycled plastics in new toy manufacturing. In this study, eighty-four toys purchased from large Europe shops were screened for bromine using XRF, and 11 of those with bromine content higher than 500 µg/g were further analyzed for ten PBDEs using GC-MS/MS. All samples had some PBDE content, and BDE-183 and -209 were detected in 10 of 11 samples. The summed PBDEs concentration in all toys ranged from 0.0002 to 106820 µg/g (median ± standard error: 27631 ± 11185 µg/g). The most abundant PBDE was BDE-209, detected at concentration ranging from <LOD to 105000 µg/g (24600 ± 10489 µg/g), accounting for an average of 91% of PBDEs in all samples. BDE-183 ranged from <LOD to 14100 µg/g (2290 ± 1302 µg/g); while other detected PBDEs were at trace levels. Nine of 11 toys had extremely high levels of BDE-209; concentration in these toys were in the mg/g range, up to 105 mg/g, or 10% BDE-209 in one toy radar. Nine samples exceeded the EU's Low POP Content Limit (LPCL) for the summed PBDEs concentration by 5 to 100 times and the Unintentional Trace Contaminant (UTC) limits for Deca-BDE by 500 to 10,000 times. The PBDEs concentration reported in this study are among the highest reported for children's plastic toys around the world, and this is a call for concern. The extremely high PBDE levels suggest direct recycling of e-waste plastics into toy components. According to the EU Toy Safety Directive, nine of the children's plastic toys analysed in this study should not be used or sold to the public. These findings highlight the gaps in enforcement of chemical safety regulations, and also jeopardize the investments in circular economy.

#### **6.05.P-Mo503 Comprehensive Risk Assessment of E-waste Dismantlers Occupational Exposure to Organophosphate and Halogenated Flame Retardants Using Monte Carlo Simulation**

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Flame retardants (FRs) are frequently added to electrical and electronic equipment to retard the development of fire. As the use of this kind of equipment significantly increases and a lot of equipment becomes quickly obsolescent, there has been a dramatic increase in the production of electronic waste (e-waste). While e-waste recycling is a necessity, the release of FRs, such as organophosphate esters (OPEs) and halogenated FRs (HFRs), during this activity can pose risks to the environment and human health. In particular, e-waste dismantling workers can suffer high FRs exposures and assessing this occupational exposure becomes a priority. The goal of this study was to provide a comprehensive risk assessment of occupational exposure to OPEs and HFRs for the largest e-waste dismantling facility in Catalonia (Spain). Risks from exposure to FRs through inhalation and dermal uptake were assessed in terms of non-carcinogenic (non-CR risk) and carcinogenic risk (CR risk) using Monte Carlo simulation. Monte Carlo simulation allowed the calculation of a probability distribution of the risks associated with FRs exposure, taking into account several exposure uncertainties. The probability distributions of the non-CR risk from FRs exposure through inhalation were below the threshold for adverse effects for all compounds except for BDE-153 and BDE-209, for which there was only a 1% probability of the risk being above the threshold. The evaluation of the non-CR risks for mixtures of FRs through inhalation also showed limited risks. The probability distribution of the cumulative exposure to all FRs suggests only a 1% probability of non-CR risks. This limited risk was driven by HFRs exposure since the probability distribution for OPEs was completely below the threshold for non-CR effects. However, since not all FRs result in the same health effects, the risks associated with exposure to FRs mixtures were also evaluated in terms of specific health end-points. For FRs with liver effects, no risks from inhalation were observed, while for FRs with neurobehavioural effects, there was only a 1% probability of health risks. Using Monte Carlo simulation it was possible to provide a comprehensive description of the occupational risks of e-waste dismantlers coming from exposure to FRs. Additionally, the risk assessment was enhanced by the evaluation of the risks from exposure to mixtures of FRs in terms of chemical groups and specific health effects.

#### **6.05.P-Mo504 Safe, Sustainable and Circular by Design Organophosphate Flame Retardants**

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Organophosphates can serve as versatile flame retardants in a variety of materials and uses. However, many organophosphate flame retardants have become notorious environmental pollutants. Tris(2-chloroethyl) phosphate (TCEP) and its analogue tris(3-chloropropyl) phosphate (TCPP) are examples thereof. While TCEP is listed as substance of very high concern under REACH since 2010, TCPP has only recently been suggested to be added to the candidate list and a new study by the US National Toxicology Program found evidence of carcinogenicity. Both chemicals are frequently found in environmental monitoring studies.

In this work we present a case study of redesigning the molecular structure of these organophosphate flame retardants for increased safety and sustainability. The design aims to increase biodegradability as a handle to prevent environmental pollution from leakage, e.g. when applied as additive flame retardant on textiles. The structure moreover contains functional groups that could allow permanent linking to matrices to be recycled with the material.

We compare this new OPFR to TCEP and TCPP, as well as the structurally intermediate alkyl phosphates triethyl phosphate (TEP) and tripropyl phosphate (TPP), which are used as polymer additives. Properties related to Persistence, Mobility, Bioaccumulation and Toxicity were predicted for these compounds using available, established, and applicable QSAR models. The poster will present the designed alternative and compare modelled properties with results of experimental testing.

For experimental testing of properties relating to function and safety, we successfully synthesized the designed alternative OPFR. Biodegradability screening experiments show an increased mineralization of the alternative compound compared to the chlorinated OPFRs and the alkyl phosphates. Acute and chronic ecotoxicity studies were performed with the algae *Raphidocelis subcapitata* and the crustacean *Daphnia magna*. Preliminary results show notably lower toxicity of the alternative compound than TCEP and TCPP. Furthermore, testing of the flame retardant function and material compatibility of the designed alternative is ongoing. To sustainably produce the alternative OPFR research is underway in our labs to synthesize organophosphates from phosphates recovered from wastewater, working towards circular, safe and sustainable OPFR.

#### **6.05.P-Mo505 Flame retardant-contaminated food contact items and toys sold on the U.S. market**

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Plastics often consist of a complex mixture of unreacted intermediates, monomers, and additives such as dyes, fillers, antioxidants, flame retardants (FRs), UV stabilizers, surfactants, and plasticizers, all to improve or modify the performance of the product. Many of these additives migrate during use and/or pose threats to human and environmental health during production and disposal. Although the EU has banned organohalogen FRs in electronics displays, few policies in the US regulate the use of FRs. Brominated compounds used to flame retard electronics have been found in consumer products purchased in Africa, Europe, and Asia due to lack of regulation and poor recycling practices. To investigate if U.S. consumer products that do not require flame retardancy are contaminated with regulated/unregulated FRs, a selection of food contact items, hair accessories, kitchen utensils, and toys was analyzed for the presence of 21 FRs. The Br content in the plastic consumer products measured with XRF ranged from 51 to 18600 ppm. BFRs and PFRs were detected in 17 of the 20 consumer products, and the BFR levels correlated well with the Br content in all but 3 cases. TBBPA was the most common FR, detected in 75% of the consumer products followed by BDE-209 (70%), 2,4,6-TBP (70%), DBDPE (60%), BDP (60%), RDP (60%), TPHP (55%) and TTBP-TAZ (50%). The highest BFR levels were found for BDE-209 with concentrations up to 11900 mg/kg detected in a sushi container. In 70% of the plastic products the levels exceed the unintentional trace contaminant limit of 10 mg/kg for decaBDE (BDE-209) as introduced by the EU. The decaBDE replacements, DBDPE and TTBP-TAZ, were also frequently detected, indicating that these compounds have also made their way into the waste electrical and electronic equipment (WEEE) stream. The finding of multiple hazardous FRs in plastic consumer products is worrying and points to the need for elimination of hazardous additives to support the move to a circular economy. Besides various BFRs previously detected in electronics displays, the finding of other FRs can be seen as a confirmation of WEEE-specific contamination as none of the consumer products analyzed needed to contain FRs. The appearance of WEEE fractions in plastic consumer products is a result of poor recycling practices and lack of FR regulation. Ongoing research is further investigating the presence of other plastic additives in these consumer products using the non-target screening approach.

#### **6.05.P-Mo506 Release hotspot identification during manufacturing of a halogen free fire-retardant additivated composite for railway applications**

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The ability of manufacturing new chemicals that are “inherently safe and more sustainable from production to end-of-life” including circularity is currently a pressing task in the context of the green transition, especially for persistent and toxic substances such as flame retardants. For this reason, in the context of the Horizon Europe project SURPASS (Safe-, sUstainable- and Recyclable-by design Polymeric systems - A guidance towardS next generation of plasticS), a new epoxy-vitrimer based composite integrating non releasable fire-retardancy moieties was developed with the aim to obtain more durable and /or recyclable material for railway applications. The SSbD framework developed by the Joint Research Center



(JRC) has been implemented in the integrated SURPASS approach and further applied in the railway sector developing lightweight composite materials. Indeed, the new composite is improving sustainability (replacing a metal-based material with a polymeric one, thus decreasing the energy consumption), increasing safety (using a halogen free flame retardant) while maintaining the desired functionality. In this work, the release hotspot identification has been conducted to determine potential release of materials (e.g., additives such as flame retardant, non-intentionally added substances and microplastics) along the life cycle of the product (from the manufacturing to its end-of-life) as Step 2 of the SSbD framework implementation. The hotspot identification has been performed by combining in a template key information on materials, products and processes involved in the case study, considering the specific data needed for workers, consumers, and environmental exposure assessment according to current regulations. The template has been filled to identify potential needs for re-design considering occupational, consumer and environmental safety in the innovation phase.

#### **6.05.P-Mo507 Where are the Chemicals? (WatCh)**

*James Delaney, Chief Scientist's Group, Environment Agency United Kingdom, United Kingdom*

Polybrominated diphenyl ethers (PBDEs) are a group of 209 man-made organobromine compounds. They have been used as flame retardants in a wide range of products including textiles, electrical and electronic equipment, and foams. The persistent and bioaccumulative properties of PBDEs, along with their potential negative impacts on humans health and aquatic life have led to commercially supplied 'penta-, octa- and decaBDE' being classified as persistent organic pollutants (POPs) under the Stockholm Convention. Furthermore, owing to the strict fire safety requirements for furniture and fittings in the UK and Ireland introduced in the 1980s, PBDEs are a particular problem in the UK environment.

The Natural Capital and Evidence Synthesis (NCES) team in the Environment Agency (EA) are looking at the material flow of POPs such as PBDEs and their impacts on the environment. To do so, project 'Where are the Chemicals?' (WatCh) is combining a series of conceptual models, including Source-Pathway-Receptor (SPR) and Life Cycle Assessment (LCA), to understand more about the material flow of PBDEs from their manufacture to disposal to their exposure to environmental receptors. Evidence outcomes of 'Project WatCh' will be used to identify regulatory interventions opportunities to explore, evidence gaps, insights to where & when pollutant risks may occur; opportunities for early stakeholder engagements, and potentially influence future policy discussions on POPs. The evidence and modelling work for 'Project WatCh' is being led by the NCES team. However, data inputs, advice, and guidance is also being provided by experts across the Chemicals Directorate in the EA.

To date, a systematic literature review has taken place, so that all research relevant to material flow analysis of PBDEs has been selected and critically appraised. Also detailed analysis of current and historical PBDE databases has been carried out, including building a data inventory. Some early results from the 'WatCh model prototype' show the levels of commercially supplied PBDE congeners 'penta-, octa- and decaBDE' that were historically manufactured in the UK between the early 1970s and the late 1990s, which allowed predictions on the levels of each congener that are currently found in different waste streams to be made.

#### **6.05.PC Flame Retardants and Regulation, Connecting Substance Grouping and Circular Economy**

#### **6.06.P How to Effectively Communicate Results From Environmental Assessment Frameworks to Support the Decision-Making Process**

#### **6.06.P-We532 Holistic multiple stressor impact assessment methodology for application to vulnerable and disadvantaged communities**

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There has been growing attention to addressing vulnerabilities of populations living under marginal conditions. These conditions may stem from socioeconomic, access to health care, physical changes in environments due to climate and other factors, and pollution. United States environmental policies are currently emphasizing the need for cumulative impact analyses to support programmatic, regulatory, and project decisions that pose additional burdens on already overburdened communities. These policies fall under the Environmental Justice initiative. Similar policy attention is emerging around the globe especially with respect to impacts of climate change, chemicals, and migrations of people. We have developed a holistic multiple stressor assessment methodology that involves participation from the impacted communities, government agencies, and business community. The methodology is organized around the concept of Human Well Being (aka One Health) and integrates human health risks with impacts upon ecosystem services. Human health risks and ecological impacts are normalized to a common metric scale so that baseline conditions and subsequent changes can be viewed collectively for individual health risks and environmental impacts; these individual risks and impacts are not added together as that can obscure important directional changes in health or environmental conditions. The integration of multiple stressors is accomplished through the presentation of results as part of discussions within and among the different groups. Thus, dialogue is essential to the process as different groups will likely perceive the results from varied cultural and personal perspectives. The proposed methodology focuses on defined populations that share common attributes, and the analytical framework allows for the designation of multiple populations so that cumulative impacts and associated gains and losses can be understood at various societal and spatial scales.

The strength of this approach is that it integrates the technical aspects of cumulative impact assessment with stakeholder engagement. Achieving the right level of detail and using various communication tools are important for reaching a shared understanding among diverse groups.

#### **6.06.P-We533 How can we explain to the public what Sick Building Syndrome is and how to defend against it?**

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Seventeen Sustainable Development Goals were adopted at the UN Summit in September 2015. UN Secretary-General Ban Ki-moon opened the summit by saying that the new agenda is a promise from political leaders to the people of the world. The goals should be understandable to everybody. If we are to meet the goals, we must understand them. Our daily behavior, our approach to other people and resources, should be influenced by these goals. An important part of this process is to be able to convey scientific issues to the general public. The issue of Sick Building Syndrome was approached from this perspective.

A project by university students with the driving question, "How can we explain to the public what Sick Building Syndrome is and how to defend against it?" was carried out in the interdisciplinary course Introduction to Toxicology. In the first part of the semester, students gained basic knowledge through lectures, interactive videos, work with professional texts and sources of information, and a practical laboratory assignment. The second part of the semester was devoted to the project itself. They worked on the Sick Building Syndrome topic in 4-member teams made up of students from different stages of their studies. The audience was specified but forms of outcomes for the real audience were based on principle "Students' voice and choice".

Project-based learning leads students to gain skills, accept responsibility and strengthen civic sense. The output of the project (video, blog, guidelines, speeches etc.) enables the scientific information to be communicated to the general public, allowing its implementation in everyday life.

#### **6.06.P-We534 Combining LCA and eLCC with a Foresight Exercise: Sustainability Assessment of Water Reuse Chains in the Mediterranean**

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Water reuse represents a valuable opportunity to tackle water scarcity in the Mediterranean area. Nonetheless, the efforts in integrating water reuse in mainstream water management practices face several challenges such as low public acceptance, high costs of reclamation and difficulties to comply with safety standards. While innovative treatment technologies emerge to achieve a safe wastewater management, comprehensive assessments to identify environmental hotspots and external costs associated with their application are still insufficient to ensure long-term viability of those initiatives and to inform decision making processes.

To identify environmental impacts and unveil the internal and external costs of reusing water, this paper develops a methodological framework to assess the sustainability of innovative wastewater treatment technologies by performing a Life Cycle Assessment (LCA) and an environmental Life Cycle Costing (eLCC).

By applying the framework on a pilot case study in Italy and upscaling the analysis to a real scenario, results shows that innovative technologies could decrease environmental impacts compared to a Business-as-Usual scenario with conventional wastewater treatment processes. Even though costs performances of innovative technologies were lower in the case of primary treatment and innovative treatment for emerging pollutants proved limited when upscaling to bigger volumes of treated wastewater.

Building from the hotspot identified in the analysis, the results from the foresight exercise identify rules enforcement on freshwater and wastewater pricing and the promotion of awareness campaigns as the key strategy to increase financial incentives and investment, to promote the reliability of solution and to increase public awareness for contributing to water reuse diffusion in the future.

Thus, the paper provides a methodological framework able to unveil the environmental impacts and costs of water reuse chains, to inform science-based policies by testing a tool to communicate results and to translate them in a set of actions to increase public acceptance and to promote the diffusion of non-conventional water resources in the Mediterranean.

#### **6.06.P-We535 Avoiding errors of the third kind: Prescriptive decision analysis to bridge the gap between public knowledge needs and scientific knowledge generation**

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Science translation methods hold promise for turning scientific observations into usable knowledge for the public and actionable interventions that can improve human health and environmental quality. Accordingly, the obverse holds equivalent merit. Often the knowledge needs of decision-makers and the public are subsumed by scientists into more expediently measured proxies. This runs the risk of committing a Type III error or getting the right answer to the wrong question. When

addressing the pressing decision information needs of society, a clear rendering of the problem context and concerns of the public is needed to bridge the gap between scientific knowledge needs and scientific knowledge generation. Prescriptive decision analysis provides a pragmatic approach to characterizing decision problems and communicating the knowledge needs of stakeholders and decision-makers to technical experts for improved data-driven environmental decision-making. Precepts of decision analysis are exemplified through the desktop application DASEES. DASEES (Decision Analysis for a Sustainable Environment, Economy, and Society) is a general decision consequence analysis application that integrates socio-economic and environmental impacts from proposed actions within a preference structure for the evaluation of alternative options and decision scenarios. DASEES facilitates an analytic-deliberative process for group decision making providing tools for risk assessment, valuation, trade-offs, and uncertainty analysis. The deliberative aspects of DASEES support issue framing, developing objectives (stakeholder values), and evaluation measures through stakeholder engagement. The analytic aspects support the integration of data, information, models, and tools identified as necessary to assess the consequences of proposed solutions. DASEES provides a framework for scientists, decision makers, and stakeholders to use relevant data and information effectively and identify additional research needed to fill knowledge gaps. DASEES allows broad inclusion of stakeholders, transparency of the decision process, and an archive of the decision information for future stakeholders who might want to review or revise the decisions in the future.

## **6.07.A Improving Chemical Regulation Through Robust Science, Data Accessibility, and Interdisciplinary Collaboration**

### **6.07.A.T-01 The implementation of the substitution principle in European chemical legislation: a comparative analysis**

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Background: The substitution of hazardous chemicals with safer alternatives is an important objective in European chemical policy, but implementation has been slower than expected. We conduct a comprehensive analysis and comparison of the implementation of the substitution principle in European regulations for pesticides, biocides, and industrial chemicals. Specifically, we examine and compare the criteria and processes associated with the identification of candidates for substitution and the assessment of alternatives.

Results: We find only minor differences in the criteria applied to identify candidates for substitution amongst pesticides, biocides, and industrial chemicals, but larger differences concerning the processes used. While all substances that are to be approved as a pesticide and biocide are systematically evaluated against the established criteria for substitution, the substitution process for industrial chemicals only focuses on those substances identified as substances of very high concern. The main reason candidates for substitution remain on the market is the lack of identified safer chemical alternatives and the insufficient consideration of non-chemical alternatives, caused, at least to a large extent, by the comparatively weak incentives provided by current regulations.

Conclusions: The systematic approach for the identification of industrial substances of very high concern (SVHC) under ECHAs “Integrated Regulatory Strategy” is much welcome. However, no final conclusion on SVHC properties or the need for regulatory action has been drawn for approximately 90% of the REACH-registered substances, as often even basic hazard and exposure data are missing. Hence, at least a screening-level evaluation of SVHC properties should become a mandatory part of the substance registration under REACH. To reduce the risk of strategic behaviour in the search for alternatives to industrial chemicals identified as SVHC, a setup in which regulatory authorities play a larger role as information and knowledge brokers should be considered. Investments in innovation as well as improved sharing of information and a better distribution of the workloads amongst European authorities might also improve the identification of safer alternatives. However, without stronger incentives, making it more costly for companies to continue using hazardous substances relative to safer alternatives, initiatives to promote substitution are likely to have limited success.

### **6.07.A.T-02 Understanding Policy Incoherence as a Driving Force for the Lock-in of Hazardous Chemicals in Automotive Plastics**

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Why does society continue to use highly hazardous substances despite knowledge of their potential for harm? Blumenthal et al. (2022) recently revealed, through a series of case studies, the “lock-in” of continued use of hazardous chemicals due to complex interactions between economic, social, technological, and political factors that reinforce the status-quo. The authors further recommended to use the “lock-in” framework to systematically understand and address the continued use of hazardous chemicals.

The current study aims to extend the understanding of lock-in by conducting a case study on the contribution of various current and prospective legal frameworks to the lock-in of hazardous additives in automotive plastics. Specifically, this study seeks to understand how different regulations jointly influence the selection and phase-out of chemical additives in automotive plastics.

It starts with identifying the key legal frameworks for a comprehensive EU-level examination, encompassing chemical safety, waste management, vehicle safety, and related policies. A comparative analysis of relevant policies, in consultation with policy-makers, researchers, and industry representatives, is conducted with an evaluation of policy synergies or potential conflicts.

The analysis highlights that strong policy incoherence exists with respect to safe management of chemicals used in automotive plastics, and that this misalignment in regulation plays a key role in re-enforcing the lock-in of hazardous chemicals. With substantial changes proposed in 2023 to management of end-of-life vehicles, this study highlights the urgency for policy makers to address existing regulatory gaps to avoid contributing to the continued lock-in of hazardous chemicals in the automotive sector.

#### **6.07.A.T-03 Mapping Substances Subject to Overlapping Regulations within the EU's Chemicals Regulatory Framework**

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The EU's chemicals regulatory framework includes regulations for everyday products such as cosmetics, food packaging and medicines as well as regulations for pesticides, biocides and industrial chemicals. Most substances entering the EU market have to be registered and assessed for potential hazard and risk, but test requirements, risk assessment processes and management options vary depending on how the substance is used. i.e. which regulations apply. Substances with several uses may therefore be assessed and regulated under more than one regulation resulting in potentially diverging assessment and management outcomes. In the EU Chemical Strategy for Sustainability, the European Commission launched the approach 'one substance, one assessment' to tackle the inconsistencies of assessing the same substance under various pieces of legislation. A more defined strategy to this approach is yet to be announced. More knowledge is needed on how the current regulatory system falls short in assessing these substances and what is needed across regulations to reach a more harmonised hazard and risk assessment approach. In this study, we have mapped out the current state by identifying substances with overlapping regulations to understand the scope of the problem and its potential downstream effect. In addition, we have performed in-depth analyses of a number of substances regulated under more than one regulation to identify inconsistencies and gaps in the risk assessment processes. Lastly, we propose steps towards a 'one substance, one assessment' approach in the assessment and management of chemicals in the EU.

#### **6.07.A.T-04 Prioritization and Identification of Emerging Pollutants for National Environmental Screening and Monitoring Programs in Norway**

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Every day new chemicals are put on the market which might potentially be of environmental concern. It is estimated that more than 350 000 chemicals are in trade today, and approximately 100 000 are in use in the EU market. However, only 22 529 substances are submitted to REACH registration. Thus, there is huge gap in information regarding chemicals that might be of emerging concern in the environment.

The Norwegian national monitoring programs for pollutants are used for assessing the impact of chemical stressors to the environment and screening programs are used for identifying emerging pollutants. However, establishing analytical methods for new chemicals is resource intensive, and only a limited number of chemicals are prioritized to be included each year. Thus, it is important to do a thorough evaluation of possible candidates with regards to both risk assessment and possible presence in the environment prior to selecting chemicals for monitoring. The lack of complete databases for chemical risk data makes the prioritization and evaluation of possible emerging contaminants challenging. Data is spread across the internet, and it is not easily compared. Conflicting or duplicate identifiers for chemicals, differences in data formats, tests, and methods for data generation are major challenges. There is also a lack of awareness among end-users of the uncertainty in data that is generated from predictions or models. Many of the tools and databases do not include information on applicability domain (AD) score or other quality indicators of predicted data.

In this project, multiple lines of evidence from different data sources, data types, and test systems are combined in a transparent assessment to give a score on persistence (P), bioaccumulation (B), mobility (M), and toxicity (T) for each chemical and the reliability of each of these assessments. This is implemented in a prioritization tool (PIKME) for filtering or ranking chemicals according to risk of being P, B, M or T and in addition according to the reliability of the assessment. This was coupled with the probability of detection in environmental samples based on possible use, previous detection, type of sample matrix, and analytical method. This allows versatility of the tool depending on the scenario to be explored: i.e., prioritization of a list of substances, screening of the most toxic in the database, selecting substances with low reliability and high predicted risk.

### **6.07.A.T-05 Data Harmonization of Contaminants in Fishery Products for Improvement in National Monitoring Programmes: the Spanish Case Study**

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One of the objectives of the Marine Strategy Framework Directive is to ensure that the levels of contaminants in seafood for human consumption remain low and within safe limits. In Spain, the legislation includes a very small number of pollutants, like some heavy metals (Cd, Hg and Pb) and persistent organic pollutants (dioxins, PCBs and PAHs). Although the EU countries have the obligation to provide data about the levels of regulated pollutants in seafood, lack of data harmonization has been observed in the past report periods, due to the fact that there is no standardized protocol for sampling and data reporting of contaminants, and part of the information is lost in this step. Thus, this work aims at identifying gaps in the monitoring programmes, in order to evaluate which points should be addressed for their improvement and to achieve a good ecological status of the marine environment.

Firstly, we obtained data from the previous Spanish monitoring campaigns and we performed a detailed literature search to compile a list of prioritized compounds. After that, a sampling campaign for the new period 2023-2025 has been designed, including selected fish, crustaceous and mollusks species from different Spanish demarcations. Methodologies based on liquid chromatography (LC) or gas chromatography (GC) coupled to mass spectrometry (MS/MS) and inductively coupled plasma mass spectrometry (ICP-MS) have been implemented for organic and inorganic pollutants, respectively.

The results helped to identify some gaps in the monitoring programmes. We found a high level of missing information and data incompleteness. In particular, data on the exact origin of the analyzed species were not always reported, although is essential to better address the needs of the different Spanish Autonomous Communities for what concerns fishery contamination. In most cases, the analyzed species did not reflect the real population consumption of seafood, and with these data we cannot provide a proper risk to human population. Another problem is the small number of contaminants currently included in national monitoring programmes. As observed, the current selection of investigated contaminants is insufficient to cover the real contamination scenario of marine environments. The evaluation of new contaminants and the associated risk assessment would be a major contribution to improve the current legislation gaps at both national and EU level and establish safe levels and good ecological status.

### **6.07.B Improving Chemical Regulation Through Robust Science, Data Accessibility, and Interdisciplinary Collaboration**

#### **6.07.B.T-01 FAIR data to support Chemical Risk Assessment and Regulation – The PARC ambition and approach**

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Risk Assessment, whether for humans or the environment, requires access to high quality data regarding both exposure and hazard of chemicals, which presents a significant challenge currently as each domain has its own methodological approaches and conventions in how to capture, store and share data, and these are not necessarily compatible (interoperable in technical terms). Thus, a major effort within PARC is being devoted to developing the working practices, and technical solutions, to enable the integration of human and environmental exposure and hazard datasets, and their use/re-use for enhanced chemical risk assessment and responsive regulation. Core to this effort is making PARC data FAIR – Findable, Accessible, Interoperable and Reusable – and machine actionable, which means that information is structured in a consistent way so that machines (computers) can be programmed against the structure. Implementation of FAIR requires intensive collaboration between domain experts who generate datasets and accompanying metadata and the technical experts who develop the support structures to enable FAIR data, such as structured and harmonized vocabularies, workflows for generation of FAIR data packages / FAIR data capture templates, tools for visualisation of PARC datasets and reporting on progress towards FAIR data. Our vision is an ecosystem of data repositories of specific data types (e.g., omics, chemical information) with an overlaid data harmonization layer to enable integration and visualization - the PARC FAIR Data Hub. To facilitate this, landscape mapping of existing FAIR enabling resources has been performed, via FAIR Implementation Profiles, extension and harmonization of ontologies for PARC domains and development of a roadmap of how to optimally implement FAIR for each domain was initiated. PARC's FAIR ambition acknowledges that we cannot foresee all potential re-uses of PARC data, but recognizes that if we link datasets and their metadata well, they will be amenable to any possible future uses. The PARC FAIR Data Hub provides workflows for PARC-produced data / metadata to enable functional linking & machine actionability to support long term findability and re-use, adding value by addressing regulatory concerns, contributing to the Digital Single Market, and ensuring positive Socioeconomic Impact from publicly funded research.

#### **6.07.B.T-02 A database on pharmaceuticals in the environment: what do stakeholders need?**

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In order to characterize pharmaceutical pollution, the potential impact of human medicinal products on the environment may be assessed by performing an environmental risk assessment (ERA). A significant amount of data has been generated but is currently dispersed across multiple data sources that are often hard to track down and find, such as regulatory ERA reports for products authorised after 2006 and databases with peer-reviewed data. Because of this, obtaining a complete overview of all available ERA data for a certain pharmaceutical is nearly impossible. To address this issue, an up-to-date and comprehensive database serving as a centralised repository for data related to pharmaceuticals in the environment (PiE) is required.

Regulators, academic and industry partners within the PREMIER project are working together to provide a transparent source of ERA data that could be used for various purposes, including scientific research and regulatory risk assessments. Besides the database, PREMIER will develop a digital assessment system (DAS), which will support performing ERAs using the available data in an appropriate and transparent way. The aim of this study is to complete the first step in the database development process, which is the identification of user requirements. To this purpose, we reached out to over 100 professionals that work on PiE. We asked them what kind of data and tools should be incorporated in the database and what features of the database they wish to use. The results showed that the work of most respondents is affected by data gaps regarding ecotoxicity, monitoring and metabolism. A lack of information on the mechanism of action of APIs mainly affects stakeholders working with authorisation, production and use of medicines. Gaps in mass spectrum data, modelled environmental concentrations and metabolism data have a greater impact on academic researchers and stakeholders dealing with environmental and water quality. Respondents also showed a clear preference for a transparent and comprehensive database, with detailed study reports preferred over brief summaries. Overall, we demonstrated that the key actors working on PiE strongly endorse the development of a database and assessment tools to support the ERA of pharmaceuticals. By providing an overview on the target users' requirements, we completed the first crucial step in the development of an accessible system to assess the environmental risk of pharmaceuticals.

#### **6.07.B.T-03 Towards FAIR Sharing of Chemical Monitoring Data**

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The European Commission's Chemicals Strategy for Sustainability proposed to establish a common open data platform on chemicals (CDPC) to facilitate the sharing, access and re-use of information on chemicals coming from various sources. The existing Information Platform for Chemical Monitoring (IPCHEM) will be one element in the future CDPC, hosting chemical occurrence data.

IPCHEM, is an already established single access point where EU, national and regional authorities, and researchers can find and share information about chemical concentrations in different media. IPCHEM contains currently nearly 600 Million concentration measurements in environmental media (air, water, soil, biota), indoor air, food and animal feed, and in humans. Our challenge is to accommodate data of different domains, originating from different regulatory or research monitoring programmes. Our aim is to make the data not only findable and accessible, but to enhance interoperability and ultimately facilitate the re-use of the data.

We achieve this by harmonising the most relevant fields which are common to all domains, while we keep information that is dataset specific and integrate it "as-is". This is important to provide some level of comparability while ensuring no information is lost and enable a transparent documentation. For transparency and reusability of the data, we also provide publicly available rich metadata describing each dataset.

In the presentation, we will explain the different steps taken in IPCHEM for improving the sustainability of the platform, our interactions with data providers and users, approaches for open and restricted data access, and quality control. We will show examples of use illustrating practical ways how the data can support answering policy and research questions in the area of chemical, environmental and health policies. Such examples include the use of pesticide residues in soil or human biomonitoring data to build indicators to evaluate the progress in reducing exposure related risks over time.

#### **6.07.B.T-04 So FAIR, So Clean: How the cleanventory Approach Provides Reliable Data for Chemical Structures Regulated in Global Trade Markets**

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With the number of regulated chemicals ever rising, there is need for a harmonized information system on regulated chemicals, especially under the newly established concept of "one substance – one assessment". As part of the H2020 project ZeroPM (<https://zeropm.eu>), a fully reproducible and open-source global chemical inventory – the "cleanventory" – is being developed. A modern database infrastructure will facilitate wide-spread use of the database, strictly following FAIR principles (Findable, Accessible, Interoperable, Reproducible). The database will be publicly available and include features for programmatic access. The public, legislators, and industry stakeholders will also have the possibility to review all the code and build their own "cleanventory" from scratch.

So far, over 990,000 inventory entries with over 225,000 unique CAS Registry Numbers and over 410,000 unique chemical names have been integrated. This amount of information could be considered "big data", but we put considerable efforts towards the quality of the data, *i.e.*, also ensuring "good data". To identify chemical structures from inventory entries, CAS Registry Numbers and chemical names are used. To convert inventory identifiers to InChI strings (*i.e.*, structural information), four freely available API services are used: PubChem (compound and substance domain), CAS Common Chemistry, CCCTE CompTox, and NCI/CADD Chemical Identifier Resolver. To identify the "most probable" chemical structure for every inventory entry (*i.e.*, the combination of CAS Registry Number and chemical name), a weighted consensus ranking approach was developed to assign each InChI strings an identification score.

Over 344,000 unique InChI strings were retrieved by the API services. After a weighted consensus ranking approach, over 126,000 unique InChI strings are identified as being the "most probable" chemical structure for the given inventory entries. This high-quality database of chemical structures on global trade markets will support the EU Chemical Strategy for Sustainability initiative by effectively enabling the concept of "one substance – one assessment" by providing robust, curated, and transparent data and workflows.

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#### **6.07.B.T-05 From Parcel to People: development of an indicator to monitor risk to residents from pesticide use in agricultural areas**

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The increase in global pesticide use has mirrored the rising demand for food over the last decades. Despite the resultant boost in crop yields, there are increasing concerns about the impact of pesticides on biodiversity and ecosystems, as well as the undeniable adverse effects on human health, especially among populations residing close to agricultural zones. This work investigates the complexities of pesticide exposure in such residential areas within France.

By integrating a range of novel spatial datasets and exposure assessment methodologies, we have developed an indicator to highlight the levels of pesticide risk faced by residents. By spatializing pesticide sales data and juxtaposing it with pesticide authorization for specific crops, we developed a detailed map depicting potential pesticide loads at the parcel level across France. This spatial distribution served as the basis for an exposure assessment, carefully modelled based on the European Food Safety Authority's guidelines, measuring the risk quotient of pesticide exposure.

The indicator, which combines the exposure map with population distribution data, provides a more nuanced understanding of pesticide risk not related to dietary exposure. Our analysis, even if centered on France, lays down an approach that could be extrapolated across the EU, supporting a data-driven approach aimed at measuring and reducing exposure to pesticides.

The comparative assessment with existing datasets, such as the LUCAS soil survey and the Treatment Frequency Index from the ADONIS map, although not entirely consistent, shows the potential and areas in need of refinement for our methodology. As Europe progresses towards its goal of halving pesticide use by 2030, our study emphasizes the need for more detailed attention to pesticide-related issues. Additionally, it highlights the critical importance of harmonized and high-resolution data in steering the region towards more sustainable farming systems. Our indicator assists policymakers and stakeholders in navigating the complex dynamics of pesticide use and its implications.

#### **6.07.P Improving Chemical Regulation Through Robust Science, Data Accessibility, and Interdisciplinary Collaboration**

##### **6.07.P-Th491 The European Network of Human Biomonitoring Laboratories: Advancing the European HBM Platform in PARC**

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Human Biomonitoring (HBM) provides information on real-life human exposures and is a useful tool for assessing the effectiveness of regulatory actions. In the last years, efforts have been dedicated towards building a European HBM platform that can support risk assessment by enhancing HBM capacities across Europe, provide relevant data to contribute to exposure evaluation and to inform the development of public and environmental health policies. As part of it, the European Network of HBM Laboratories was set up within the HBM4EU project to connect experts in analytical chemistry and HBM and support the less experienced laboratories to improve their skills.

To extend the network and better understand the real HBM analytical capacities in Europe, a questionnaire was sent to the laboratories previously included in the network, as well as to new laboratories nominated by the PARC National Hub Contact Points. The survey collected information on the laboratories' capacity to quantify different chemical groups (> 20) in various human matrices, data on instrumental techniques used and limits of quantification, among others.

Information was obtained from 108 laboratories from 27 countries. Metals is the group of substances more widely analysed (60%), followed by bisphenols (32%), phthalates (31%), organochlorine pesticides (31%) and PFAS (28%). The substances less analysed are dioxins and furans, and musks (5.6% and 0.9%, respectively). Blood, urine and hair are routinely analysed but the analysis of chemicals in less common matrices (e.g. amniotic fluid, saliva), as well as in of innovative samples (e.g. dried blood spot samples and volumetric absorptive microsampling) is also reported. The results of the first analysis of the collected data have been conveyed to the final selection of exposure biomarkers to be analysed in the PARC General Survey.

A further analysis will allow to identify the real capacities and skills of the European laboratories performing HBM analyses. This will be the basis for building the future activities of the network in terms of training and interactions, as well as to identify urgent needs to be addressed, such as the development of new analytical methods. The European Network of HBM Laboratories is an important step to achieve a common approach to HBM in Europe, can improve HBM capacities at country level and is a perfect scenario for opportunities and collaboration among European HBM laboratories.

#### **6.07.P-Th492 Strengthening Collaboration for Next-Generation Environmental Risk Assessment (NG-ERA): A Horizon Europe PARC Platform and Process for Governance of Scientific Coordination**

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The Partnership for the Assessment of Risks from Chemicals (PARC) provides a European-level partnership for regulatory actors and researchers in the EU to address the challenges faced in human health and environmental risk assessments (ERAs). These challenges include assessments of low-dose effects of chemicals; indirect and combined effects of multiple chemicals. Climate change and habitat loss on biodiversity decline are currently not explicitly addressed in PARC but could be included if a platform is developed. Further, fragmented regulatory assessments under the current substance-by-substance approach strongly suggest that innovative and integrative approaches across regulatory sectors are needed. To this end, a cross-disciplinary project is under development to strengthen the collaboration within PARC and with external projects, aiming to develop and facilitate the implementation of Next-Generation ERA (NG-ERA). A strong engagement of European regulatory agencies in PARC should facilitate regulatory relevance and uptake. As a partnership at the EU level, PARC has the potential to provide a platform to facilitate collaborations for a more efficient use of research resources and to enhance the scientific foundation of new approaches and tools developed for regulatory adoption.

The objective of this project is to establish a collaborative platform and processes for the governance of scientific coordination in NG-ERA. This involves mapping existing efforts, identifying gaps, and to develop a framework and processes for cross-project collaborations. The project aims to be linked to an ongoing activity in PARC, that explores collaborative approaches for NG-ERA of pesticides: *Risk assessment to support and promote efficient overall protection of biodiversity*. Please contact us to become a partner in the development of this platform to strengthen the effort to integrate data, knowledge and areas of expertise and to better protect our environment.

#### **6.07.P-Th493 OECD QSAR Assessment Framework: Advantages and disadvantages of a tool designed to assess and ultimately strengthen predictive NAMs**

**Paul Thomas, Floriane Larras, Franklin Bauer, PhD, Gaspard Levet and Maxime Edelblout, KREATiS SAS, France**



NAMs are an essential piece of the upcoming Green Deal if Europe is to move to a “toxic free environment” and safe and sustainable by design products. *In silico* NAMs up to now have obtained less recognition than their *in vitro* cousins in this sprint towards faster and more animal free methodologies. And yet, *in silico* NAMs are ultimately the fastest, cheapest alternative to laboratory methods and potentially the most elucidating, particularly so, when they are based on mechanistic interpretation.

The ambition of the fairly recent QSAR Assessment Framework (QAF) is to help regulators across the OECD member states to better assess the likely prediction capacity of QSARs by using a reflective step-by-step check list to arrive at a consensus result. There is no doubt that as we are submerged by QSARs of all types and levels of quality, the QAF will both increase overall acceptance and use of these methods by the regulatory community as only the most robust QSARs will survive assessment.

On the other hand, the QAF further limits the wider applicability and potential of QSARs, restricting their capacity to reduce animal testing and their use as tools that can further the science in a way that empirical studies alone cannot.

In this poster we will provide demonstrations of the advantages of QAF. We will also prepare case studies where mechanistic QSARs have the necessary functionality to assess certain situations already well recognized within the science of ecotoxicology but QAF does not contain sufficient flexibility to allow recognition of this fact. An example is where toxicity is greater than the water solubility limit for certain (hydrophobic) substances for certain endpoints and where “unambiguous algorithms” cannot be obtained. Other examples will be elucidated and remedies proposed.

#### **6.07.P-Th494 Shifting Towards NAMs Based Risk Assessment: A Korean Case Study**

*Dawoon Jung, Yunsun Jeong, Sung Yong Jung and Ranhee Kang, Korea Environment Institute (KEI), Korea, Republic of (South)*

Under the Act on Registration and Evaluation of Chemicals in Korea, all chemical substances manufactured in or imported into Korea must be registered. It is estimated that over 1.402,000 tests are required for about 17.096 substances by 2030. Although the Ministry of Environment has set the goal of conducting 60% of all chemical risk assessment using alternative testing methods, the use of alternative methods in chemical hazard assessment remains less than 10%. Therefore, a careful examination of the current state of the technological development, legal base, and regulatory application of new approaches methods (NAMs) is necessary in order to enhance the institutional advancement of animal alternative testing in chemical risk assessment. In this study, we analyzed the current legal, systematic, and research status of NAMs in Korea. At the same time, we conducted detailed interviews with stakeholders to identify major issues in the current regulatory application process. As a result, we proposed a strategy to promote NAMs in chemical risk assessment in Korea. First, the technical and regulatory expertise of alternative test methods must be enhanced through organizational separation of development and validation, and regulatory application. Second, we need to reprioritize the methodologies that warrant funding by gathering input from experts as well as government and industrial stakeholders. At the same time, administrative support during process of development-validation-international acceptance (e.g., OECD test guideline) has to be strengthened. Finally, the regulatory application of alternative test methods should be enhanced by strengthening technical and administrative training for the practitioners. The results of this study will contribute to the advancing and institutional implementation of alternative methodologies for animal testing in the chemical safety assessment in Korea, fostering a paradigm shift toward safety assessment centered on next generation risk assessment. This study was conducted with the generous support of KEITI (RS2023-002-15309) and KEI (RE2023-018).

#### **6.07.P-Th495 Differences and similarities between levels of protection of the aquatic ecosystem under different EU chemical regulations - Part I: surface waters**

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Environmental regulation of chemicals in the EU was implemented to protect the environment against potential adverse effects. As a chemical may be suitable for different uses, different regulations may apply to the same chemical. While all uses may impact the same environmental compartment, one substance may have several different regulatory threshold values for the protection of surface water ecosystems. For many years this had no consequence because there were no points of contact between the different regulations that could have flagged inconsistencies. During the last decade though, the comparison of concentrations measured in surface water from monitoring campaigns for pesticides to thresholds values showed that, depending on the selection of the threshold value, the resulting risk may be acceptable or not. This created confusion among scientists and in the public domain, where the expectation prevails that there could only be one threshold concentration that can be labelled “safe” based on current scientific knowledge. This discrepancy has led the European Commission to set the objective of the Chemicals Strategy for Sustainability to move towards the ‘one substance, one assessment’ approach, the aim being to improve efficiency, effectiveness, coherence and transparency of hazard assessments across all relevant legislation. To support this EU strategy and to consolidate and strengthen the chemical risk assessment, the EU funds a partnership under Horizon Europe (PARC). Here we report from a project aimed to elucidate whether the risk assessments of a chemical under different regulations indeed differ in their level of protection. For this purpose, we compared the scopes and protection goals of

the various legal frameworks as well as the underlying data and methodologies. We compiled and compared regulatory requirements for active substances for authorization for plant protection products (EC 1107/2009), biocidal products (EC 528/2012), veterinary medicines (EU 2019/6) and REACH as well as effects assessment for retrospective risk assessment under the EU WFD (EC 2000/60). The investigation focused on differences in the procedure, the protection goal, the conceptual approach and resulting management measures. We used imidacloprid as a case study to pinpoint congruence and differences between the assessments for the aquatic compartment. This presentation complements the results for the sediment compartment, which are presented in a separate contribution.

#### **6.07.P-Th496 Differences and Similarities Between Levels of Protection of the Aquatic Ecosystem under Different EU Chemical Regulations - Part II: Sediments.**

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Environmental regulation of chemicals in the EU was implemented to protect the environment against potential adverse effects in water, sediment, soil and air caused by their use. While all uses may impact the same environmental compartment, the environmental assessment requirements differ between environmental compartments and, for a same environmental compartment, it may differ from one regulatory framework to another. As chemicals may be suitable for different purposes, different regulations may apply for the same chemical. For many years, this had no consequence because there were no points of contact between the different regulations that could have flagged inconsistencies. During the last decade though, the comparison of concentrations measured in surface water from monitoring campaigns for pesticides to threshold values showed that, depending on the choice of the threshold value, the resulting risk may be acceptable or not. This discrepancy has led the European Commission to set the objective of the Chemicals Strategy for Sustainability to move towards the ‘one substance, one assessment’ approach, the aim being to improve efficiency, effectiveness, coherence and transparency of safety assessments across all relevant legislation. To support this EU strategy and to consolidate and strengthen the chemical risk assessment the EU funds a partnership under Horizon Europe (PARC). Under PARC, a project is implemented aiming to elucidate whether the risk assessments of a chemical under different regulations indeed differ in their level of protection for the aquatic ecosystem. We compared the scopes and protection goals of the various legal frameworks for the sediment compartment as well as the underlying data and methodologies. Specifically, we compiled and compared regulatory requirements for active substances for authorization for plant protection products (EC 1107/2009), biocidal products (EC 528/2012), veterinary medicines (EU 2019/6), and REACH as well as effects assessment for retrospective risk assessment under the EU WFD (EC 2000/60). The investigation focused on differences in the procedure, the protection goals, the conceptual approach and resulting management measures. This presentation complements the results for imidacloprid regarding the surface water compartment, which are presented in a separate contribution.

#### **6.07.P-Th497 Phenanthrene: A Regulatory Journey in the European Union**

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Phenanthrene is a ubiquitous polycyclic aromatic hydrocarbon (PAH) which has been subjected to rigorous regulatory scrutiny within the European Union’s Registration, Evaluation Authorization and Restriction of Chemicals (REACH) framework. Here we provide a concise summary of the regulatory assessment of Phenanthrene under REACH and highlight key aspects of the multi-year process.

Phenanthrene is a constituent of Coal tar pitch high temperature (CTPHT) which was classified as a substance of very high concern (SVHC) in 2009, due to the properties of its main constituents. In this assessment Phenanthrene was identified as a constituent in CTPHT with very persistent & very bioaccumulative (vPvB) properties. Subsequently, in 2018 an SVHC dossier for Phenanthrene was submitted by the French Member State Competent Authority. The SVHC submission was agreed by the Member State Committee in 2018 and Phenanthrene was included on the Candidate List of substance of very high concern for authorization.

In 2021, a judgement by the European Court of Justice (ECJ) concluded the lack of manifest error in the assessment of Phenanthrene. It also highlighted that substances on the candidate list could, upon review of new information, be de-listed. The court indicated that the European Chemicals Agency (ECHA), where necessary, must carry out a re-examination of the SVHC conclusion when new & relevant information is provided. This decision effectively identified a novel regulatory mechanism under Article 58 (8) of REACH.

In early 2022, a data package with new lab-based information was provided to the European Commission’s Directorate General for Internal Market (DG GROW) under the novel mechanism identified by the ECJ. This communique requested the reassessment of the Phenanthrene SVHC conclusion. DG GROW, in defining this novel process, formally requested that ECHA assess the information for its novelty and relevance. In late 2022, ECHA concluded that the new information did not impact the conclusion of Phenanthrene as a SVHC and therefore no further steps were necessary.

There are several important aspects of the Phenanthrene regulatory journey which are worth highlighting, including that the chemical is not registered under REACH which limits the potential regulatory mechanisms of action, the ECJ's identification and formalization of a mechanism to submit new, relevant information, and the novel process developed by DG GROW and ECHA for the submission of said new information.

#### **6.07.P-Th498 High level screening strategy to determine impact of new CLP hazard classes on chemical portfolios**

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A central ambition of the EU Green Deal is the transition towards a toxic-free environment. New hazard categories under the Classification, Labelling and Packaging (CLP) Regulation (EC) No 1272/2008 came into force in April 2023 related to Endocrine Disruption, Persistent/Mobile/Toxic (PMT), very Persistent/very Mobile (vPvM) and the existing PBT/vPvB (B stands for Bioaccumulative) classifications. For all substances placed on the market in the European Union these categories will need to be implemented as per 1<sup>st</sup> November 2026. It can be foreseen that substances classified for one or more of these categories will be restricted or banned from the EU market in the future. This can have a major impact on the marketed products portfolio and supply chain security of companies.

ERM has developed a strategy to identify chemicals within a chemical company's portfolio that potentially fall under the new hazard categories. The portfolio is evaluated following a high level, stepwise approach taking into consideration all data available for a substance in the public domain, starting from data published by authorities worldwide. These data are evaluated and if no conclusion can be drawn on the possible hazard classification, *in-silico* and read-across data from similar substances are also used to aid the evaluation. Finally, all data are evaluated in a weight of evidence approach and, if possible, a conclusion on the classification is drawn. The classification is ranked based on the reliability of the collected data.

Substances used in the Bayer Consumer Health European product portfolio were identified for screening that either had the potential for classification and/or were present in important products for the business. The overarching aim was to develop an understanding of the associated business risks of potential classification for Bayer Consumer Health and the associated supply chain, determine the potential impact and feasibility of product reformulation and ultimately develop more sustainable products that continue to allow consumer self-care.

This screening approach represents a first step to identify chemicals affected by the new hazard classes under CLP. It is an essential step to mitigate business risks but, more importantly, it is a tool to increase the sustainability of the chemical portfolio of a business. An overview of the strategy employed and major learnings in its implementation to a consumer health ingredient portfolio are presented.

#### **6.07.P-Th499 REACHing for solution: case of chemical regulation and regrettable substitution**

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Chemical regulation is generally regarded as a major driver in the substitution of hazardous chemicals. As a substance of concern is subject to regulatory scrutiny and/or going through regulatory process of being identified as a substance of very high concern (SVHC), industry can respond through the use of alternatives.

The REACH Regulation aims to ensure control of the risks from hazardous chemicals to human health and the environment and encourage innovation and use of alternative chemicals or technologies where available. Processes such as authorization and restriction are key drivers in this. For example, authorization aims to encourage the substitution of hazardous chemicals with safer alternatives or technologies where available and possible.

Despite best efforts by both the industry and policymakers, instances of substitution with unintended consequences have been reported. But very little is known about the role that chemical regulation plays in this. This study therefore aims to stimulate discussion on this perspective by addressing the following questions (i) does chemical regulation unintentionally drive regrettable substitution? (ii) in what ways does regulation exacerbate regrettable substitution?

These questions have been answered through engagement with stakeholders using online questionnaire and interviews. Stakeholders including policymakers, industry, NGOs, academics and consultants were engaged. Feedback was gathered and analysed.

Preliminary finding(s) and opinions from the stakeholders are "banning a group or class of substances may lead to unintended consequences e.g. losing important chemicals from the supply chain", "the need for policymakers to provide clear and workable guidance" and "lack of regulatory certainty is anti-innovation and investment".

The findings will help contribute to policy development, ensuring the aim of regulation to protect human health and the environment from risks of harm from hazardous chemicals is achieved.

#### **6.07.P-Th500 German Court Judgement: The Right to Environmental Information Grants Access to Environmental Risk Assessment Data and Studies from the Human Medicinal Product Authorisation Procedure**

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The pollution of pharmaceuticals in the environment is of concern and risk mitigation measures should further be developed. Environmental risk assessment (ERA) is part of the European authorisation procedure of veterinary/human medicinal products since 2005/06, but the availability of these data for the field of environmental protection is limited (Schwonbeck et al. 2021).

In a legal dispute, the plaintiff applied for access to data and studies on environmental risk assessment for selected medicinal products for human use from the national competent authority, relied on §4 I German Environmental Information Act. As the regulatory authority did not grant access after hearing the marketing authorisation holder (MAH) and invoked intellectual property rights, among other things, the Administrative Court of Cologne, Germany, made a final decision in this case (ECLI:DE:VGK:2023:0713.13K5068.18.00):

The competent authority is required to grant the plaintiff access to the complete environmental risk assessments, i.e., all documents of the submitted dossier required under Annex 1 part I. module 1. point 1.6. "environmental risk assessment" of Directive 2001/83/EC and information for each of the selected substances (medicinal products).

By clarifying the accessibility of ERA data and ERA studies, the German court has made a groundbreaking decision with European implications. The decisive legal bases for this are anchored in European law, and a detailed legal analysis has already been provided in the "Oelkers papers" (Oelkers & Floeter 2019; Oelkers 2020; Oelkers 2021a; Oelkers 2021b) and the "Feasibility Study of an Active-substance-based Review System ('Monographs') and Other Potential Alternatives for the Environmental Risk Assessment of Veterinary Medicinal Products Final Report" for the European Commission (Schwonbeck et al. 2021).

The scientific and environmental law implications of this judgement are presented.

Oelkers, Kim, Floeter, Carolin (2019): DOI: 10.1186/s12302-019-0256-3.

Oelkers, Kim (2020): DOI: 10.1016/j.yrtph.2019.104571.

Oelkers, Kim (2021a): DOI: 10.1016/j.scp.2021.100386.

Oelkers, Kim (2021b): DOI: 10.1016/j.scp.2021.100399.

Schwonbeck, S., Breuer, F., Hahn, S., Brinkmann, C., Vosen, A., Radic, M., Vidaurre, R., Alt, J., Oelkers, K., Mezler, A., Floeter, C. (2021): DOI: 10.2875/94477.

#### **6.07.P-Th501 Environmental Fate Data of Chemicals from Regulatory Dossiers – Harmonisation of Public Assessment Reports across different Regulation?**

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In the EU, chemicals are regulated differently depending on their use (chemicals, biocidal products, medicinal products for human or veterinary use, plant protection products). However, for all substances the environmental fate and effect data provided by industry is usually considered confidential. Consequently, across different regulatory frameworks either limited or quite detailed information and summaries on adverse effects or behaviour in the environment are published as endpoints in (European) Public Assessment Reports (PAR or (E)PAR). When it comes to metadata associated with environmental fate endpoints (e.g., amount of non-extractable residues (NER), minimum and maximum DT50, DT90, transformation products, use of radioisotopes, used kinetic model, goodness of fit, etc.) such data are in most cases not publicly available.

The legal situation for veterinary medicinal products (VMP) has recently changed (Regulation (EU) 2019/6), while the regulation for medicinal products for human use is currently being revised ([https://health.ec.europa.eu/medicinal-products/pharmaceutical-strategy-europe/reform-eu-pharmaceutical-legislation\\_en](https://health.ec.europa.eu/medicinal-products/pharmaceutical-strategy-europe/reform-eu-pharmaceutical-legislation_en)). In both cases, a monograph system for ecotoxicological and environmental fate data is to be introduced. It is particularly important for the scientific community that the information in the monographs is much more detailed than in the EPAR or PAR. Read-across studies and data comparability studies require a high amount of detail that exceeds the one that is stated in these assessment reports.

To illustrate this need for harmonisation and comparability of data, we will present results from a comparison of data from publicly available batch simulation studies in soil, water/sediment and water (OECD 307, 308 and 309) and address important details that are crucial for answering scientific questions. Data was taken from public sources such as the REACH database (ECHA registration dossiers), from the peer-reviewed scientific literature and grey literature.

### **6.07.P-Th502 Perspectives on Increasing Complexity in the Environmental Fate and Ecotoxicology Regulatory Space**

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Current environmental fate and ecotoxicology challenges relevant to PPP registration include lack of agreement on risk assessment (RA) approaches for complex active substances (AS) (UVCB, inorganic, naturally-derived), difficulty interpreting higher-tier approaches, implausible study designs, and uncertainty in guideline implementation timelines. These obstacles often result in EFSA data gaps which multiply issues to be resolved by MS during product registrations. In contrast, recently published guidance present elaborate RA frameworks for birds, mammals, bees, residues in drinking water treatment, and soil exposure modelling without addressing the existing challenges. Thus, there is a twofold problem of increased scientific complexity coupled with unresolved regulatory uncertainty.

While reasoned, science-based approaches for evaluating AS are urgently needed, we see a growing misalignment between guidance and obstacles faced when conducting RA. Increasing regulatory complexity without first resolving existing challenges and evaluating unanticipated consequences does not serve the common goal of sustainable agriculture in the EU. Particularly for biopesticides, inorganic, and UVCB substances, fit for purpose guidance is a priority. Reasoned guidance is integral to ensuring plausible study designs; e.g. the new bee guidance proposes an unreasonable number of treatment fields for a honeybee study (up to several hundred). This highlights the necessity of feasibility checks and tailored calculation tools to reliably conclude on further testing.

Case studies applying new and proposed approaches to existing AS would be an excellent first step to better understand the impact of new guidance. Further, increased opportunities for transparent exchanges for evaluations of non-standard compounds would both lessen the regulatory burden and increase opportunities to find timely, collaborative solutions. Lastly, there is an urgent need for guidance that facilitates reasoned RA of complex compounds while leaving flexibility for substance-specific approaches.

In conclusion, increasingly complex assessment frameworks could magnify uncertainty in EFSA evaluations which could then significantly increase workload for MS already at capacity and limit their ability to evaluate novel approaches. It is imperative that notifiers and evaluators are equipped with appropriate tools to support evaluation of AS and products and achieve the goal of sustainable crop protection in the EU.

### **6.07.P-Th503 Improving Implementation of the Stockholm Convention and other Global Governance Frameworks for Chemical Management in the Republic of Korea**

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The Republic of Korea ratified the Stockholm Convention in 2007, and as a subsequent action, 'Persistent Pollutants Control Act' was enacted. According to this law, every five years, the Ministry of Environment develops a master plan for the implementation of the Convention. While this national plan encompasses most of the necessary fields for implementing the Convention, achieving some goals, such as preemptive response to the decisions of the Convention, are challenging within the current national policy timeframe. For instance, although there have been updates to the list of persistent organic pollutants (POPs) and POPs candidates, the preparation in Korea for responses (e.g., ratification, national chemical production status and risk evaluation) is progressing slowly. Recent global initiatives on chemicals, including the Science-Policy Panel on chemicals and wastes and a New Plastic Treaty (plastic-associated chemicals), will require national responses and actions in Korea such as undertaking a gap analysis of relevant policies and authorities. However, to the best of our knowledge, there has not been sufficient national-scale progress on those initiatives. To address the aforementioned issues, we propose establishing a national scientific-policy panel in Korea. This panel would be a format of governance consisting of national POPs experts (scientists), the relevant chemical industries, regulators, and policymakers. The role of panel would involve conducting relevant and valuable assessments, offering policy-relevant advice, cooperating with the international scientific communities, participating as the scientific advisory to the national representatives in COPs, and effectively disseminating the results of its work to audiences across various sectors who can take meaningful action. By establishing a national science-policy panel, Korea can actively contribute to the decision-making processes of global governance frameworks on chemicals with the accumulated knowledge, opinions, and experiences. A more proactive role of panel should be explored in the long-term, for instance, proposing chemicals relevant to the Korean population to be monitored in the national biomonitoring scheme, which would be a notable example of science-based decision making. Given the growing emphasis on science-based decision-making in the chemical management, Korea should take this opportunity to lead and proactively contribute to global initiatives.

### **6.07.P-Th504 HEALTH-BASED LIMIT VALUE REPOSITORY – CURRENT STATUS AND FUTURE CHALLENGES**

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Health-based limit values (HBLV) are used in scientific and regulatory contexts to protect human health and the environment from adverse effects after exposure to different chemical substances and mixtures. They are usually defined as the highest dose or concentration at which no adverse effects are expected or at which the risks are considered acceptable in the light of the benefits of its use. Scientific HBLV are derived by using experimental data and further uncertainty factors, while regulatory HBLVs may consider further modification due to socio-economic, technical feasibility or political aspects in the derivation

process. Currently, limit values are accessible on separate online platforms in different structures and formats. These limit values are often showing limited detail of metadata, validity, reliability and a low level of transparency on their derivation process and thus complicating their reuse.

Within the project “Designing EU repository of health-based limit values and collating information for the first version of the repository” (ENV/2021/OP/0019), commissioned by the European Commission’s Directorate General for Environment (DG ENV), Novamechanis Ltd. and Fraunhofer ITEM were awarded to develop the first outline for an EU-uniform repository of human, animal and environmental HBLVs in a centralized and structured platform. As part of this project, 128 HBLVs were systematically identified and mapped, including background information and associated metadata, from the databases EUCLEF, Open Food Tox, the EU Pesticide database and a questionnaire conducted and provided by DG ENV. Furthermore, the user patterns, needs and requirements of the prospective users of the planned repository were requested and analysed in an online survey. The collected HBLVs from the mapping process and assessed knowledge from the survey lead to a first list of limit values and metadata to be included in the future repository.

The project showed that the current system for reuse of HBLV needs further improvements and a new repository is very welcomed by all user groups. In conclusion, the planned centralized and structured HBLV repository would contribute to the reuse and harmonisation of HBLVs and thus to the EU objectives of the ‘One substance, one assessment’ approach in the Chemicals Strategy for Sustainability of the European Commission.

#### **6.07.P-Th505 The Mixture Allocation Factor & Options to Address Unintentional Environmental Mixtures Across Substance Regulations**

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Across Europe, the prospective environmental risk assessment (ERA) focuses on single chemicals and is conducted under separate regulatory frameworks, i.e. for plant protection products, biocides, REACH chemicals, as well as for human and veterinary pharmaceuticals. However, a variety of chemicals enter the environment together from different sources, irrespective of their legal framework, leading to co-exposures that may cause joint effects. While requirements to address intentional mixtures (e.g. product formulations) and aggregated exposures (i.e. emission of one substance from different uses) exist in some substance-oriented legislations, obligations to assess and regulate unintentional mixtures are missing. In the European Commission’s Chemicals Strategy for Sustainability, a so-called MAF (Mixture Allocation Factor) was proposed as a generic regulatory management tool for REACH to address risks due to the co-exposures and subsequent combined effects of chemicals. However, this approach might not work for all substance-oriented legislations and is far from being the “one and only” solution to effectively address mixture risks. Its applicability, challenges and benefits have to be evaluated for each relevant legislation. We discuss the challenges for the assessment and regulation of mixture risks via generic and specific approaches and outline different options for the different substance-oriented legislations. Options are for example: the MAF as a generic approach, specific mixture assessments for defined scenarios via component-based approaches, or the prioritization of mixture drivers and substance exceedances followed by regulatory measures. Moreover, the development of an accessible data basis and communication tools for users as well as discussions on societal needs and benefits are needed. Overall, overarching approaches across prospective and retrospective legislations are warranted to approach the Zero Pollution Ambition to finally benefit from a reduction of chemicals.

#### **6.07.P-Th506 A Gap Between Risk Assessors and Risk Managers in the Case of Pharmaceuticals in the Environment**

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The emission of pharmaceuticals into the environment can have a serious impact on the exposed ecosystems. Therefore, it is difficult to comprehend the existing gap between pharmaceutical and environmental legislation. Currently, there is no direct legal link between the conclusions from environmental risk assessment (ERA) (as a part of pharmaceutical authorization) and the management of the environmental impacts when the product reaches the market.

When the outcome of the ERA indicates a risk for the aquatic compartment and risk mitigation measures are set in the summary of the product characteristics (SPC), no legal obligations are in place to communicate this information to authorities responsible for environmental management.

In Europe, various chemical regulatory regimes (covering industrial chemicals, pesticides, feed additives, biocides, and pharmaceuticals) are in place (EU COM, 2018). The transparency of protected data related to use, fate and behaviour and the environmental effects substantially differs within different regulations. While comprehensive and detailed ecotoxicological information is provided within legislative frameworks covered by REACH and BPR (industrial chemicals, biocides), pesticides and feed additives, the same data for pharmaceuticals are scarce, not easily accessible and often limited just to outcomes of the procedure (Oelkers, 2020).

Environmental data from the pharmaceutical authorisation procedure are relevant for policy under the Water Framework Directive (WFD)(Directive 2000/60/EC), the Urban Wastewater Treatment Directive (WWTD) (Directive 91/271/EEC, as amended by Directive 98/15/EC) and the Industrial Emissions Directive (IED) (Directive 2010/75/EU), where this piece of information is used for the derivation of environmental quality standards (EQS) or emission limit values.

The gap between regulatory frameworks should be bridged through enforceable legal provisions. The monograph system or/and the chemicals strategy objective to move towards “one substance, one assessment” could serve as mechanisms for transparently reporting the relevant data (The European Commission, 2020). These legal provisions should also foresee that national authorities responsible for environmental management may provide feedback on environmental issues due to the use of pharmaceutical products, e.g., monitoring data, to the National Competent Authority for medicinal products and/or European Medicines Agency.

#### **6.07.P-Th507 Enabling circular non-toxic supply-chains.**

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Today's production and consumption systems are putting a strain on natural resources at a pace that surpasses society's ability to manage the challenges that follow. In attempts to organize its response to anthropogenic pressure actors globally are developing circular economy strategies as means of sustainable use of resources. An important but often disregarded part of the circular economy is hazardous chemicals. Such chemicals are part of everyday products and when recycled they will inevitably be part of the circular loop of resources. The realization of a non-toxic circularity has several challenges, such as global supply chains and fragmented chemical regulation. This paper discusses how to enable circular non-toxic supply chains with a focus on goal conflicts and synergies connected to circular chemicals management, issues of transparency and traceability in supply chains, and managing difficult trade-offs and knowledge gaps concerning circularity and end-of-life. The study is exploratory and builds on semi-structured interviews and reference group discussions with respondents working with chemicals management and circular economy. The results show that a transition to a circular non-toxic economy is impeded by insufficient legislation and policy incoherence. Moreover, there is low transparency and traceability of chemical content in global supply chains, which in turn leads to a low control of chemical content in materials and products and difficulties for actors to make informed decisions. This may lead to a circulation of legacy and hazardous chemicals, thereby significantly increasing consumer and environmental exposure. However, designing products and materials non-toxic from the start enables several circularity synergies and opportunities for a sustainable use of resources. The results also illustrate how a hazard-based approach to risk management further enhances circular supply chains and safer consumer products. This study enhances the discussion on the circular economy by discussing the barriers, challenges and synergies concerning hazardous chemicals in the circular loop of resources. Further, it demonstrates relevant steps towards the realization of circular non-toxic economy use is necessary in a transformation to a society that meets the current environmental and resource challenges.

Keywords: Circular economy, non-toxic, supply chains, resource use, chemicals management

#### **6.07.P-Th508 Impurity assessment in the EU from an (eco)toxicological perspective. How do we know if an impurity is relevant and if it enhances the (eco)toxicity of an active substance?**

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In light of the most recent EU strategy for the sustainable use of chemicals, it is evident that in order to comply with its more complex objectives, there is a need for implementation of chemical regulation from a broader perspective. Thus, interdisciplinary work is needed for an increasing number of assessment procedures. The assessment of impurities is a clear example in which the joint efforts of experts from different fields like Chemistry, Toxicology and Ecotoxicology are required for addressing the issue in a holistic approach.

The assessment of impurities has been a regulatory requirement for many years. However, low attention seems to have been given to the relevance and impact of the impurities, present in the technical material together with the active substances, on their ability to alter the (eco)toxicity. Under the sustainability framework the attention is now increasing, but for now, there is still little feedback from authorities available on the process, possibly because the assessment relates to the confidential information of the dossiers. This lack of feedback is hindering the adoption of a clear and concise evaluation procedure.

Based on our experience with several submissions and first reactions from authorities, we come to present an approach, in which we detail the assessment steps for each impurity of potential (eco)toxicological concern identified in the 5-batch analysis study. For the individual steps, we will describe the inputs required from the (eco)toxicological experts as well as the interactions with the other expert fields involved in the process by using a workflow scheme, in which the gaps and uncertainties of the evaluation are highlighted. Further, we discuss the relevance and usability of each source of information supporting the (eco)toxicological assessment (studies, databases, in silico predictions) in the context of this stepwise approach. With this work, we aim to provide answers to the questions of whether a given impurity is relevant or not and if the impurity is increasing the (eco)toxicity of the active substance considering the proposed specifications of the technical material.

### 6.07.P-Th509 The CATs are out of the bag: Experiences to date with Critical Appraisal Tools

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In October 2022, EFSA (European Food Safety Authority) published a proposal document for the use of Critical Appraisal Tools (CATs) for non-standard ecotoxicology studies. The document outlines the theory behind the development of the CATs, why they are considered relevant, and the seven types of studies they have been created for. CATs for two additional study types are also provided as part of the revised EFSA 2023 bird and mammal guidance document.

The premise of these tools is that they provide a framework for the evaluation of non-standard studies (i.e. where guidelines are not available), in theory making the evaluation of such studies easier, and making outcomes more consistent across studies and regulators. Although application of these CATs is currently not mandatory, the inclusion of two of these in the (yet to be noted) EFSA 2023 bird and mammal guidance implies that EFSA are keen on their implementation. However, it is not yet clear when mandatory application of these tools will be required, nor if regulators will begin to use them in the meantime on newly performed, or existing, studies.

Since the release of the CATs, CEA has had experience in evaluating studies and updating study reports using the requirements in these tools. The aim of this poster is to provide examples of the use of CATs for certain study types; to summarise changes to reporting and/or study plans which may be needed in response to the CATs; and to highlight potential implications for the ecotoxicology risk assessment from their use.

### 6.07.P-Th510 Are Current Regulatory log Kow cut-off Values fit-for-purpose as a Screening Tool for Bioaccumulation Potential in Aquatic Organisms?

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Persistent, Bioaccumulative and Toxic (PBT) and very Persistent and very Bioaccumulative (vPvB) are regulatory hazard categories that have been set to manage the possible risks to humans and the environment from these chemicals. In industrial chemicals regulations, their aquatic Bioaccumulation potential is usually assessed first with a screening based on the octanol/water partition coefficient ( $K_{ow}$ ). However, current log  $K_{ow}$  cut-off values triggering classification, categorisation and/or further fish bioconcentration testing are not harmonised worldwide, and they have never been assessed for their regulatory relevance. In this study, the experimentally determined log  $K_{ow}$  and fish bioconcentration factors (BCF) of 532 chemicals were compared. While the analysis underlined the robustness of using log  $K_{ow}$  as a screening tool (5/532 were false negatives; log Kow: non-bioaccumulative, but BCF: bioaccumulative), it also demonstrated the conservatism of the cut-offs used worldwide. Indeed, many chemicals were deemed potentially Bioaccumulative based on log  $K_{ow}$  when a fish bioaccumulation test showed no concern (false positives), therefore, leading to unnecessary use of vertebrate animals. Our analysis shows that the log Kow cut-off could be increased to 4.5 in all regions for all purposes without leading to a reduced protection of humans and the environment.

### 6.07.P-Th511 Taking into account data quality and uncertainty to guide informed chemical substitution of PMT/vPvM substances

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Measures are needed to protect water sources from substances that are mobile, persistent and toxic (PMT) or very persistent and very mobile (vPvM). PMT/vPvM substances are used in a diverse range of applications, including cosmetic products. Our previous research showed that the combined application of the essential-use and functional substitution concepts can be used to phase out these substances in cosmetic products, due to e.g. the availability of safer alternatives identified through an alternatives assessment. Existing experimental data was used in this assessment, which was primarily obtained from REACH registration dossiers. However, aspects with regards to the limitations of the experimental data were not considered. Furthermore, it will be important to include results from non-standardised tests as well as considerations of data quality and uncertainty to help the decision-making process when evaluating chemical alternatives. In addition, the use of in silico predictions is key to fill in data gaps as experimental data is often not available. The reliability of these in silico models and their outcomes also necessitates careful evaluation as, for example, the models need to be scientifically valid, relevant for regulatory purposes and applicable to the chemical structure of interest. Our objective was to develop and implement an uncertainty evaluation for the hazard assessment of chemical alternatives, consequently increasing the transparency of alternatives assessments. Our proposed evaluation system draws inspiration from established frameworks like the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) and Transparency, Reliability, Accessibility, Applicability and Completeness (TRAAC). Finally, we aimed to explore how both experimental and in silico data can be effectively combined in the pursuit of moving towards safer chemical alternatives and contribute to the better management of chemicals.

### 6.07.P-Th512 Revisiting Data Quality – Illustration with Fish BCF Data

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Data quality is an important but often overlooked aspect of ecotoxicological data. Current practice on data quality assessment



typically involves the implementation of domain / phenomenon-specific screening protocol based on presupposed data quality criteria that could be traced back to various analytical or theoretical aspects of the experiment in question. The general principles of classifying data quality have been developed and applied for over two decades. Despite the widespread implementation of data quality screening, the validity and effectiveness of such approach – which can lead to exclusion of certain measurements from subsequent meta-analysis or modeling applications – has not been carefully examined. This study aims at providing a first look at the effectiveness of data quality screening based on presupposed data quality criteria using the well-established fish bioconcentration factors (BCF) database as an example. The original data quality tags associated with the fish BCF measurements were adopted without modification and/or re-evaluation though these were used as pivots to differentiate and compute compound-specific log BCFs. A suite of analyses were used to examine how log BCF values changed as a function of their data quality tags or criteria status. No statistical differences in log BCF between overall low-quality and high-quality measurements were observed for close to 90% of the examined chemicals. Among the 6 presupposed data quality criteria for BCF, at least 3 were found to exhibit minor influence / bias on log BCF values. Tree models of deviation in log BCF also revealed the lack of common hierarchical elements in the error structure of the deviation. This result was also consistent when tracking the difference from high-quality log BCF as a function of number of data quality criteria violated. Finally, it can be demonstrated that simple averaging without data quality differentiation produces statistically identical log BCF as those obtained with high-quality measurements without any noticeable dependence on BCF or log  $K_{OW}$ . These unexpected results suggested that the applied data quality assessment was, overall, not as effective as supposed. A number of plausible explanations were highlighted and discussed. Implications of data quality assessment on benchmarking and modeling environmental chemistry and ecotoxicology are discussed.

### 6.07.P-Th513 Reporting Chemical Data in the Environmental Sciences

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Environmental science, including chemistry and toxicology, is a highly interdisciplinary field incorporating researchers with various backgrounds and expertise such as: environmental chemistry, toxicology, atmospheric sciences, biology, data sciences, health science, plus physical, organic, analytical, and biological chemistry. One key aim of this scientific intersection is to understand the behaviour of chemicals in the environment and the interactions of chemicals with humans and other organisms. This interdisciplinary aspect is critical to addressing issues of chemical pollution, environmental sustainability, and health. However, to be effective in tackling these issues, standardized methods for reporting chemical data must be used. This is becoming increasingly important as reliance on and use of computational analysis methods, and particularly cheminformatic tools, grows. At the same time, the number of chemical structures in databases is growing exponentially. Although there are methods for clearly identifying chemicals, from database specific identifiers to names and structural information, most environmental chemistry and toxicology journals have few or no requirements and expectations for reporting chemical data. Thus, correctly identifying chemicals in literature can pose a significant challenge. This poster will review the different methods available for chemical identification and provide minimum reporting requirements for identification of chemical species using structural information and provide resources on how to access and use this information. The purpose is thus to provide recommendations on how to report chemical data in a Findable, Accessible, Interoperable and Reusable (FAIR) manner within environmental science, chemistry, and toxicology. This will broaden the scope and applicability of environmental research to empower community efforts to tackle issues of chemical pollution and sustainability in a comprehensive manner.

### 6.07.P-Th514 Curated Mode-of-Action Data and Effect Concentrations for Chemicals Relevant for the Aquatic Environment

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Chemicals in the aquatic environment can be harmful to organisms and ecosystems. Knowledge on effect concentrations as well as on mechanisms and modes of interaction with biological molecules and signaling pathways is necessary to perform chemical risk assessment and identify toxic compounds. To this end, we developed criteria and a pipeline for harvesting and summarizing effect concentrations from the US ECOTOX database for the three aquatic species groups algae, crustaceans, and fish and researched the modes of action of more than 3,300 environmentally relevant chemicals in literature and databases. We provide a curated dataset ready to be used for risk assessment based on monitoring data and the first comprehensive collection and categorization of modes of action of environmental chemicals. Authorities, regulators, and scientists can use this data for the grouping of chemicals, the establishment of meaningful assessment groups, and the development of *in vitro* and *in silico* approaches for chemical testing and assessment. This work is published and available via Scientific Data (Schulze et al. 2023) and ZENODO (10.5281/zenodo.7983816).

### 6.07.P-Th515 Towards Safer Insect-Based Feed and Food: Evaluating Metal Uptake and Elimination in the Larvae of Black Soldier Fly

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The advantages of the use of insects for food and feed are manifold. Insects are rich in protein, lipids and other nutrients and characterized by a highly efficient bioconversion capacity. Another added value in the view of circular bioeconomy is their potential to utilize a broad spectrum of food sources in the rearing facilities, including residual biomass, rather than to compete with highly relevant food commodities (wheat, soybean, etc.). Before using alternative feedstocks (bio-waste), ensuring the safety of these insects as food and feed is essential. This study aimed to evaluate the uptake and elimination of the common contaminants: cadmium (Cd), arsenic (As), and lead (Pb) by the larvae of *Hermetia illucens* (black soldier fly, BSF). Two-phase bioaccumulation studies were employed: a 5-day uptake phase (larvae were fed on contaminated substrate), followed by a 5-day elimination phase (larvae were fed on clean substrate). The substrate used was the Gainesville diet spiked with CdCl<sub>2</sub>, NaAsO<sub>2</sub>, and Pb(NO<sub>3</sub>)<sub>2</sub> at the concentrations corresponding to 2 mg/kg of Cd and As, and 10 mg/kg dry substrate of Pb, based on the EU regulatory benchmarks for maximum allowed concentrations in feed. The control consisted of organisms exposed to clean substrates in both phases. The photoperiod of 16h/8h (light/dark), and temperature of 25±2°C were used during the exposure. The animals were sampled daily, followed by the 12-hour depuration, lyophilization and quantification of the metals by inductively coupled plasma mass spectrometry. The obtained data sets from the two phases were used in toxicokinetic modelling. The kinetic bioaccumulation factors were 1.21, 1.45 and 6.13 for As, Pb, and Cd, respectively. Our results showed that BSF larvae accumulated the three metals in concentrations above the levels permitted by existing legislation (2 and/or 10 mg/kg dry substrate). The highest uptake rate was that of Cd, followed by As and Pb. However, larvae reached internal concentrations recommended for safe use as food for other animals after 2 (As), 4 (Pb) and 5 (Cd) days in clean substrate. We propose the implementation of this depuration period after exposure to bio-waste, which may vary depending on the classes of contaminants involved. Toxicokinetic studies are adequate tools for the safety assessment of insects as feed and food, and they also define the conditions that assure their sustainable use in bio-waste management.

#### **6.07.P-Th516 Towards Safer Insect-Based Foods: Evaluating Benzo(a)pyrene Uptake and Elimination in Yellow Mealworms**

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Currently, feeding the world's population sustainably and safely is crucial. Edible insects are at the forefront of sustainable food production, with some species showing the capacity to become vital tools for circular economic strategies. Given insects' ability to convert organic material into valuable products, it is imperative to understand the hazards associated to potential contaminants in their feed. While metals and mycotoxins have received major attention in research, the study of Polycyclic Aromatic Hydrocarbons (PAHs) remains scarce. PAHs are relevant due to their widespread sources, toxic nature and unexplored bioaccumulative potential in edible insects. Moreover, current EU legislation concerning PAHs is limited as there are no current legal thresholds for PAH concentrations in feed.

This study aims to evaluate the uptake and elimination kinetics of Benzo(a)pyrene (BaP) in the Yellow mealworm (*Tenebrio molitor*) as a representative compound of PAH contamination. Organisms were exposed to substrates spiked with different BaP concentrations (0.03, 0.3, and 3 µg BaP/g substrate) for 21 days (uptake phase), followed by 21 days in a clean substrate (elimination phase). Organisms were sampled at 0, 1, 3, 7, 14, 21, 22, 24, 28, 35 and 42 days, and at each sampling time, organisms followed a 24-hour depuration period previous to BaP measurements in sampled organisms. This work showed that insects can eliminate BaP from their bodies and verified a dose-dependent accumulation of BaP. After 21 days of exposure, larvae accumulated BaP, with Kinetic Bioaccumulation Factors of 1.93, 3.27, and 2.09 for 0.03, 0.3, and 3 µg BaP/g, respectively, exceeding the lowest threshold in food regulation (0.001 µg/g in infant formulae). However, following a 13-day period in a clean substrate, organisms pre-exposed to a concentration of 0.03 µg BaP/g eliminated BaP and fell under the regulatory values (half-life (DT50) of 4.19 days). Differing, organisms exposed to 0.3 and 3 (DT50s of 4.3 and 10.2, respectively) still retained BaP levels over the legal limit after the 21 days of the elimination phase.

In summary, the edible insect food industry requires the establishment of clear PAH guidelines for insect feed while aligning with existing human consumption regulations. This research highlighted the necessity for scientifically regulating PAHs in edible insects.

#### **6.07.P-Th517 Clarifying Regulatory Needs to Advance the Environmental Risk Assessment of Chemical Pesticides**

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The EU Partnership for the Assessment of Risks from Chemicals (PARC) aims to develop next-generation chemical risk

assessment to protect human health and the environment. Within PARC, the Task 6.4.4 “*Risk assessment to support and promote efficient overall protection of biodiversity*” is dedicated to advance the regulatory prospective environmental risk assessment (ERA) of chemicals, with an initial focus on chemical pesticides. This will be undertaken within the current regulatory framework of pesticides as well as beyond, with the ambition of striving towards a systems-based approach for ERA.

Currently, five main projects are carried out under Task 6.4.4, of which the “clarifying regulatory needs” project plays an overarching role for the four research-based projects (i.e. exposure, effects, benchmarked ERA and landscape-based ERA), as it aims to ensure the regulatory and policy relevance of the research conducted under Task 6.4.4. This will be achieved by: (1) tailoring the research questions addressed in Task 6.4.4 to relevant policy targets and strategies under the European Green Deal; (2) identifying actionable scientific knowledge; and (3) delivering knowledge, data and scientific tools for ERA that can be taken up smoothly in a regulatory context. As such, the “clarifying regulatory needs” project will guide the research, and foster the development and implementation of scientific tools proposed under the current Task 6.4.4 projects.

To achieve this goal, an iterative and collaborative knowledge-building process will be followed. Thereby, the views and perspectives of relevant stakeholders will be collected and discussed at the European, national and regional levels through a series of online surveys and workshops throughout the duration of PARC. The feedback gathered will enable to further assess the strengths and weaknesses of the current ERA framework for pesticides, and explore avenues to advance it. This poster will report on the main outcomes of the initial round of stakeholder engagement, which will focus on risk assessors and is scheduled to take place in Q1-Q2 of 2024.

### **6.07.P-Th518 Three Pesticide Indicators Based on Sales Data, Exposure, Ecotoxicity and Risk Mitigation Measures to Show Trends in the Risk Potential of Pesticides**

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In recent years, a number of indicators with different levels of complexity have been proposed to evaluate trends in plant protection product (PPP) risks, each with advantages and disadvantages. Very simple approaches like the Harmonised Risk Indicator (HRI) have been criticised for largely neglecting crucial factors such as exposure and ecotoxicity.

The environmental risk indicators presented here (Korkaric et al. 2022, Korkaric et al. 2023) aim to provide a more holistic perspective on a national level, specifically designed for Switzerland. Based on national sales data of PPP, the approach integrates exposure, ecotoxicity, and the estimated impacts of risk reduction measures. In this way, an indicative trend analysis of the risks for organisms in surface waters and terrestrial habitats, as well as of the risk of groundwater contamination is obtained.

The basic idea is to weight the outcome of a regulatory risk assessment with an estimate of the treated area, obtained from the amount sold annually and a mean application rate. Risk mitigation measures are considered in terms of their effectiveness, the relevance of the affected exposure pathway concerned, and the degree of implementation. For the surface water compartment, all parts of this scheme were implemented, taking into account product specific mitigation measures as well as general point source mitigation measures. For semi-natural habitats (terrestrial ecosystems outside the treated fields) a simplified scheme is currently used. A simplified approach is also used for the groundwater indicator, based on the treated area and the estimated concentration of transformation products from regulatory groundwater exposure assessments.

In this poster, we present the implementation of the approach as well as the evolution of these indicators for the period 2012-2022.

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### **6.07.P-Th519 Synthetic pyrethroids and water quality**

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Synthetic pyrethroids are a group of chemically related compounds used as pesticides and pharmaceuticals. The synthetic pyrethroids share similar properties such as a tendency to adsorb to particles, sediments and animal tissues. They are also extremely toxic to aquatic invertebrates and fish.

A recent model study showed that three synthetic pyrethroids, deltamethrin, esfenvalerate and lambda-cyhalothrin, are responsible for 90% of the total environmental impact by plant protection products from open cultivation in Dutch surface waters.

Also, most synthetic pyrethroids are difficult to measure in water at the level of their environmental quality standards such as from the Water Framework Directive (WFD-EQS). They are therefore 'non-evaluable'. Because of their high potential impact in waters and their non-evaluability, synthetic pyrethroids are considered as problematic substances in water quality management.

The authorisation criteria for most synthetic pyrethroids used in Plant Protection Products (PPP) are orders of magnitude higher than the environmental quality standards for surface water. This may lead to water quality standards being exceeded. Authorisation criteria for synthetic pyrethroids used in biocides are also higher than the EQS, but differences are smaller than for PPPs. In the case of synthetic pyrethroids that are both used in PPP and biocides, authorisation criteria for biocides are significantly lower than for PPP.

These differences between regulatory thresholds are the consequence of regulatory frameworks having different protection goals and methods, and different ways of collecting and using scientific data. The imbalance between authorisation criteria and WFD-EQS for synthetic pyrethroids leads to different insights into their effects on surface water quality and hampers a consistent approach towards minimising the impact of these substances. A more uniform assessment of these substances at the European and national level could be a solution to this.

Existing measures to reduce the environmental impact of synthetic pyrethroids in the Netherlands include Emission Reduction Plans (ERPs) by manufacturers and measures in the framework of Integrated Pest Management, such as using alternative methods of pest control or more environmentally friendly insecticides. Fewer environmental emissions of synthetic pyrethroids to surface waters would potentially result in a significant improvement of chemical and probably the ecological .

#### **6.07.P-Th520 PCBs an Emerging Pollutant of Concern...?**

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Polychlorinated biphenyls (PCBs) are man-made chemicals that were once extensively manufactured in various industrial applications as commercial mixtures. PCBs were subsequently acknowledged as environmental contaminants. Health risks associated with PCB exposure prompted a worldwide discontinuation and the implementation of stringent regulations governing their production, utilization, and disposal. Most research related to PCBs concentrates on assessing the congener components found in commercial mixtures, or marker substances representing those mixtures (e.g. 17 PCBs 28, 52, 101, 118, 138, 153 and 180). Biomonitoring studies are reporting declines in environmental PCB concentrations because they only focus on a few PCBs linked to historic commercial PCB mixtures. However, studies that have looked for all 209 PCBs are detecting elevated concentrations of by-product PCBs that are not linked to commercial mixtures.

By-product PCBs are PCBs that are inadvertently generated during a variety of different chemical processes. They have been referred to previously as inadvertent PCBs, incidental PCBs (i-PCBs) and non-Aroclor PCBs. These PCBs have been identified as the dominant source of exposure in some residential properties in the U.S. They have been estimated to contribute between 4-50% of the total PCBs found in marine mammals in the North Atlantic Ocean. They are also dominating PCB profiles in Chinese soils and sediments.

There is currently a discrepancy in current legislation that means the importance of these PCBs is only set to continue. Current legislation in the U.S. permits the release of higher concentrations of PCBs produced as by-products, than PCBs that were produced intentionally. There are limits on the concentrations of inadvertently produced PCBs in commercial products, however these may not be stringent enough. We estimate that more PCBs may be currently produced as by-products than were produced in the 1970s during peak aroclor production. Is it therefore possible that we need to designate PCBs as an emerging pollutant of concern?

#### **6.07.P-Th521 Exploring Practice, Challenges, and Priorities for Human Health and Ecological Risk Assessments in Indigenous Communities in Canada: A Multi-sector Survey**

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Environmental pollution poses unique and complex risks to many Indigenous communities in Canada, often because of unique exposure pathways and impacts on culture, spirituality, language, and traditional food systems. However, to date institutionalized human health and ecological risk assessment (RA) approaches have not been developed or implemented with these unique contexts in mind. Through amendments to Canada's Environmental Protection Act, there is regulatory interest in developing new approaches to RA, and thus an opportunity to increase their relevance for the Indigenous communities in which they will be ultimately applied. Therefore, we conducted an anonymous online survey of those who are involved with RA in Indigenous communities in Canada, including community members and the variety of sectors they collaborate with, to: first, understand RA practice in Indigenous communities; second, explore challenges with conventional RA methods and compare these across sectors; and third, gather perspectives on the development of new approaches. A total of 38 completed survey responses were received (14.1% response rate). For our first objective, RA practice professionals spanned Indigenous community environment and health offices (21%), Indigenous governments (8%), federal and provincial governments (21%), and academia (45%), and had a wide range of experience levels with risk assessment work, ranging from less than a year to

over 20 years. For our second objective, risk communication was seen as the most challenging step of the risk assessment process (71% of responses “difficult”), with key differences between respondents working within and outside of communities. There was agreement amongst nearly all respondents that time (86%), cost (76%), and resource availability (86%) were moderate to severe problems. In response to our third objective, relatively few respondents (16%) had heard of New Approach Methodologies for RA, while 76% of respondents (and 100% of community-based respondents) agreed there was a need to develop new and improved RA approaches. Participants emphasized cumulative risk assessment; risk communication; and Indigenous leadership and autonomy as priorities for new RA methods. Overall, there were notable differences in perspectives amongst sectors; improved communication and multi-sector collaboration may advance RA practice. Future research will focus on the design and development of RA approaches led by collaborating communities.

#### **6.07.P-Th522 Phase out of lead in hunting ammunition – a perspective from an EU Member State: Denmark**

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Session 6.09

Lead is a widespread metal that society has used for millennia, and its toxicity has been recognised for almost as long. Yet, it is only within the last half century that society has actively sought to phase out the use of lead, for example in petrol and paint, for human health reasons. Hunting ammunition spreads lead in the environment where it serves as a major source of poisoning and constitutes a health risk to people consuming game meat. Hunting remains today the largest single source of dispersed lead in ecosystems. Mass-produced types, where lead is replaced with non-toxic, safe and effective alternative metals are available.

Denmark was one of the first countries to enact regulation of lead ammunition. After increasing awareness of the risks of lead shot to waterbirds in the 1970s, the first regulation of lead shot for sport shooting was introduced in 1981. In 1986 lead shot for hunting in wetlands was banned, followed by a ban on lead shot in upland hunting in 1993 and a total ban on lead shot in hunting and on trade and possession in 1996. From April 2024 the regulation has included also lead in hunting rifle ammunition.

Here we highlight the perspectives for the Danish regulation of lead ammunition to materialize in a *de facto* stop of dispersal of lead from ammunition to nature and ecosystems and to what degree this will benefit nature conservation, human health, and the sustainability of hunting. Studies show large compliance with the present regulations of lead gunshot and, consequently, low lead concentrations in key species of predators and scavengers. Furthermore, game meat lead concentrations have decreased to a safe level in Denmark compared to countries with no restrictions. Over the last five years, Danish hunters have increasingly and voluntarily switched from lead to non-lead in rifle ammunition and the perspectives for solid compliance with the 2024 regulation are very promising.

In Denmark there is around 170.000 hunters comprising app. 3 % of the total population thus one of the highest densities in Europe. Hunting is inspired by both Scandinavian, German, French, and British traditions. So even if Denmark is small in terms of land it represents traditional hunting forms and wildlife management in most of Europe. Denmark’s lead-free hunting journey can therefore be an inspiring example of how Europe and other continents can build a non-toxic future.

#### **6.07.P-Th523 REACH registered Per- and Polyfluoroalkyl Substances (PFAS) in firefighting foams: Is ecotoxicological data sufficient to assess environmental risk?**

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PFAS, or Per- and Polyfluoroalkyl Substances, are synthetic chemicals employed in various industries for their specific properties such as resistance under extreme conditions or water and grease resistance. These substances have become a growing source of concern due to their persistent and bioaccumulative nature leading to widespread contamination of soil, water, and ecosystems. They are also associated with potential health risks including developmental issues, immune system dysfunction, and an elevated risk of certain cancers.

The apprehension surrounding PFAS has led to the introduction of various regulations, navigating from individual cases to a broader, group-oriented approach based on structural definition.

ECHA recently proposed a restriction of their use in firefighting foams with an objective of a 13 000 tonnes emission reduction within 30 years addressing consequences of their potential to bioaccumulate, their persistence and their toxicity for human health.

Because environmental exposure and its effects on wildlife are out of scope of this ongoing restriction, the question of ecotoxicological risk arises.

In an attempt to make this evaluation for firefighting foams PFAS currently on the market, we identified active registrations under REACH (EC1907/2006). For each of these substances, the ecotoxicology data set was analysed for both aquatic and air breathing organisms. Data availability in disseminated registration dossiers was compared to literature.

Among the list of 78 PFAS used in firefighting foams proposed by ECHA in its restriction proposal, only 10 are registered under REACH at tonnages up to >1000 T.

Although acute aquatic toxicity data is available in every analysed dossier, data relevant for risks assessment is incomplete. Literature survey revealed a rich data set for historical PFAS whereas data for firefighting foams PFAS currently in use is scarce.

#### **6.07.P-Th524 Assessing Awareness and Compliance With Fish Consumption Advisories on the Upper Hudson River: Implications for Risk Management of the Hudson River Superfund Site**

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Polychlorinated biphenyls (PCBs) are a known environmental pollutant. From 1947 to 1977, General Electric (GE) dumped approximately 1.3 million pounds of PCBs into the upper Hudson River from their factories in Hudson Falls, NY and Fort Edward, NY. In order to prevent exposure to PCBs via fish consumption, the New York State Department of Health (NYSDOH) issues fish consumption advisories throughout the Hudson River. The NYSDOH also instates a “Do Not Eat” order for fish caught in the upper Hudson River - from Glens Falls, NY to Troy, NY. There is limited research regarding the effectiveness of such advisories in preventing the consumption of contaminated fish in a Superfund site. We surveyed 150 anglers who were actively fishing in the Hudson River from Hudson Falls, NY to Troy, NY. Participants were surveyed regarding their knowledge of the fish consumption advisories and consumption of fish from the upper Hudson River. Anglers were found to have an incomplete awareness of the advisories, and a number of individuals chose to consume fish that they caught. Awareness of advisories was inversely correlated with fish consumption, although a number of individuals who were aware of the advisories still chose to consume. Age, race, and possession of a fishing license were associated with awareness of fish consumption advisories. Institutional controls appear to have somewhat of a positive impact in preventing exposure to PCBs. However, given that a subset of individuals chose to consume fish caught in the upper Hudson River, these controls are not fully effective in their goal of preventing dietary exposure to PCBs. Remediation strategies for contaminated waterways should consider incomplete adherence to fish consumption advisories. This research provides unique insight into the efficacy of fish consumption advisories as a regulatory policy aimed at limiting human exposure to chemicals in fisheries.

#### **6.07.P-Th525 Developing UK-specific chemical emission futures within the global context**

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Chemical emissions result from numerous environmental and climatic, technological, socioeconomic and political drivers. The use of exploratory scenarios is one approach to develop understanding of how drivers may evolve, interact and affect emissions.. By linking the broader context of socioeconomic and political context to future emissions, policies and practices can adapt to and/or mitigate impacts. The challenge is to identify a framework that covers all relevant drivers at the appropriate scale, matching higher level drivers with context-specific facts. The UK Shared Socioeconomic Pathways (UK-SSPs) describe five contrasting socioeconomic pathways for the United Kingdom that coherently provide a range of challenges. They provide resources including narratives, systems diagrams and 50 semiquantitative socioeconomic variables, and have already been used to develop quantitative spatial projections including land use and population.

For chemical emission estimation, further scenario enrichment to quantify the variables directly driving emission quantities and patterns (proximal drivers). Some quantitative drivers are already available as UK-SSP outputs, but others, such as from the supply of raw materials for production, chemical import and export trends, demand for specific chemicals, and societal attitudes to chemical use, need to be developed. Using an internal participatory approach, we have derived approaches to estimate trends in the future inputs of metals to UK soils, and ongoing work is expanding this to involve external policy-related stakeholders in the estimation of emission trends for a range of substances, for the projection of future trends in water quality across the UK. The process that we have developed for deriving trends in proximal drivers for a chemical or group of chemicals involves (i) identification of the proximal drivers, accounting for all sources of the chemical or chemical type, and (ii) elucidation of historic trends in the proximal drivers, using a multi-actor participatory approach.

We will describe the approach to proximal driver trend estimation, and show examples of derived trends and discuss the key points, advantages, potential disadvantages and possible developments and improvements to this emission trend derivation approach.

#### **6.07.P-Th527 Health Risk Evaluation on Recycled Plastics: the Case Study of Dermal Exposure of Plasticizer from the Actual Recycled Plastic Products**

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In the context of the Circular Economy, plastic recycling has been drawing attention. One of the problems is that there have been few risk assessments for contained substances that can be found in recycled plastics, include both intentionally and unintentionally added. In the absence of quantitative risk assessment, recycled plastics might be required excess safety and/or recycled plastics with ensuring sufficient safety might be supplied in market. As the results, the circular amount of recycled plastic would not be increase.

Focusing on the specific issues of recycled plastics, we need to develop and suggest the appropriate methods of chemical analysis and risk assessment. Then we launch the first case study consisting of the following steps.

1. Collecting the samples of pellet for recycled plastic and interview their recycling manufacture.
2. Sample analysis to identify and quantify the chemicals which is regulated and/or restricted to control chemical risk. From these results, we aim to suggest analytical methods appropriate for risk assessment on recycled plastics.
3. Exposure assessment by material flow analysis and model simulation under the various use/application cases.
4. Risk assessment:  $f(\text{Toxicity, Exposure, Scenario})$ .

We have asked several recycling manufactures to provide sample pellets and interview. From 10 manufactures, we received about 50 sample pellets and 1 sample product (plastic holder for nail clipper).

For some of these samples, we analyzed and measured contained substances by mass spectrometry and X-ray fluorescence analysis. Prioritizing the detected chemicals and metals, we selected phthalates as our first case study and examined their acceptable/tolerable daily intakes for risk evaluation. Then we assumed exposure scenario that plastic holder for nail clippers caused their dermal exposure and set parameters such as contact time (minute) and transfer coefficient (cm/s), and we evaluated their human health risk.

Exercising and summarizing these case studies based on the actual recycled plastics we aim to publish Safety Assessment Guidance for various stakeholders in the recycled plastics supply chain who are responsible for quality control, risk management and product liability.

Acknowledgement: The part of this study is based on results obtained from a project, "Circular Economy System", in Cross-ministerial Strategic Innovation Promotion Program, Cabinet Office, Japan commissioned by Environmental Restoration and Conservation Agency.

#### **6.07.P-Th528 Unveiling Hidden Threats: Non-Conventional Endpoints In Environmental Risk Assessment For Enhanced Contaminant Impact Understanding.**

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Environmental risk assessment (ERA) is an essential tool for evaluating the potential ecological effects of chemicals. However, current ERA practices rely heavily on conventional endpoints such as mortality, growth, and reproduction. Although these endpoints provide valuable information, they may not be sufficient to protect wildlife populations when considering chemicals with specific modes of action (MoAs), such as neurotoxic compounds, or when present in concentrations below threshold values but over a long time. With an increasing number of new chemical compounds and new MoAs, there is an urgent need to modernise current ERA practices to adequately protect the environment and its inhabitants. Incorporating non-conventional endpoints (e.g., biochemical, physiological and behavioural endpoints) can aid in identifying potential adverse effects of contaminants that cannot be detected with conventional ones. The current paper aims to review and discuss the importance of non-conventional endpoints and if and when it is necessary to include them in ERA in addition to conventional endpoints.

Literature research was performed to assess under which, if any, circumstances non-conventional endpoints may prevail over conventional ones. For this purpose, the term conventional endpoint was defined by summarising all endpoints used in OECD and ISO guidelines. In a subsequent step, the term non-conventional endpoint was determined using the standartox and SCOPUS database and the VOSviewer software to construct a bibliographic map and sort out the emerging endpoint groups scientifically validated for ERA. Connections between these endpoints and temporal trends for their use were also analysed. After identifying the essential non-conventional endpoints, a more in-depth literature search was conducted, selecting several case studies comparing conventional and non-conventional endpoints to examine their importance based on the chemicals' MoA, exposure conditions and ecological protection goals. We recommend using non-conventional endpoints, such as behavioural endpoints (e.g. activity, phototaxis), to detect chemicals with specific MoAs that can lead, for instance, to neurotoxicity, olfactory impairment or endocrine disruption or can harm the environment at low, environmentally relevant

concentrations due to undetected effects with non-monotonic dose-response curves. Finally, we will present our constructed framework, summarising our suggestions to improve modern ERA.

#### **6.07.P-Th529 Common Approach for Setting Environmental Self-Classifications for Metals and Metal Compounds**

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The methodology and data treatment approaches that are applicable for the determination of the environmental classification of metal and metal compounds have been outlined in various international and European regulations and official guidance documents. The classification approaches presented in these documents, however, have been interpreted in different ways by industry and regulators. Different classification strategies, for example, were followed in the recent regulatory harmonized environmental classifications for Copper and Lead.

To promote a common interpretation of these provisions for self-classification, Eurometaux organized an Environmental Classification Workshop that was held in Brussels in December 2023, aiming for the development of a common self-classification strategy among metal associations and metal industry. The proposed classification strategy applies the concept of “the best science available” in relation to the regulatory frameworks and promotes that inorganic substances that have been assigned to the same environmental hazard category for a specific environmental endpoint indeed have comparable hazard profiles.

A correct categorizing of substances requires that they are assessed against the same benchmarks. For environmental classification purposes these benchmarks should be acute and chronic adverse effects under standardized test conditions for a number of standard surrogate species that represent three key trophic levels (algae, crustaceans, fish). Deviating from these principles by considering additional information for data-rich substances, such as other taxonomic groups or non-standard test conditions, may lead to substances with similar hazard profiles receiving different classifications. A similar logic can be followed when considering the appropriate effect-parameters.

The environmental classification scheme includes a tiered methodology to derive acute and chronic Ecotoxicity Reference Values for the metal toxicophore based on relevant and reliable ecotoxicological information. The final tier applied depends on the data-richness of the metal (use of geometric mean values, bioavailability normalization, SSD-application, pH-banding).

The application of the Environmental Classification scheme for metals and their compounds, alloys and related complex materials developed at the workshop will ultimately lead to a harmonized application of the environmental classification rules for these substances.

#### **6.07.P-Th530 Beyond ‘Surface Activity’: Addressing Challenges of Standard Chronic Aquatic Toxicity Testing with Surfactants**

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Surfactants are high production volume chemicals, often registered under the highest tonnage band (> 1000 tonnes per year) under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Thus, chronic aquatic toxicity testing is a standard information requirement for the production, import, and sale of these substances in the EU. In the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD 23) ‘surface activity’ is identified as a property of a difficult test substance, and surfactants are identified as being associated with a range of testing difficulties due to this property. However, surfactants (e.g., alcohol ethoxylates, alkyl amines, alpha olefin sulfonates, etc.) are often associated with other properties which make them ‘difficult to test’. For example, surfactants can be surface active and poorly soluble/hydrophobic, toxic at low concentrations, photodegradable, biodegradable, adsorbing, ionized, multi-constituent, and/or UVCB substances. This can make it difficult to carry out successful chronic aquatic tests using standard methodologies, even with due consideration of recommendations in OECD TG 23. All of these factors can result in test failure (leading to animal welfare issues like excessive use of vertebrate fish) and/or generation of effect values that may not be useful for quantitative risk assessment or classification and labeling. As such, there is a need to identify pragmatic and tangible strategies through which chronic aquatic test requirements for these surfactants can be addressed. This will become critical as REACH registrations extend to polymers, and the scope of registration increases.

To address this issue, a project was developed through ERASM (<https://www.erasm.org>) to leverage existing data and the (eco)toxicological expertise of industrial scientists and research organizations to address the issue of conducting chronic aquatic toxicity testing on surfactants. This project aims to: 1) generate robust definitions of ‘difficult to test surfactants’ based on substance properties; 2) provide robust evidence of challenges encountered during chronic testing using standard methodologies; and 3) critically review the applicability of available alternative chronic test methods for these substances.



Overall, this will yield guidance that can be employed during chronic surfactant testing, improving the likelihood of test success and utility of derived effect data.

#### **6.07.P-Th531 Evidence on the effects of Flame Retardant substances at ecologically relevant endpoints: A Systematic Map Protocol**

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Background: Flame retardants are a diverse group of chemical substances that are widely used in products, such as furniture, textiles, electronics and building materials, to prevent or slow the development of fire. Flame retardant (FR) substances are known to pose a risk to human and environmental health, with complex pathways of exposure and contamination. Once released into the environment, some FR substances are known, or predicted to have direct and indirect effects on long term survival, development, physiology and behaviour across a range of species, including humans and wildlife. Over time, FR substances have become the focus of many environmental and (human) health risk assessments. Systematic Evidence Maps (SEMs) have been identified as an underutilised tool for chemical risk assessment, providing a core and reliable approach to evidence-based toxicology. A list of potential FR substances has been developed (i.e. Bevington et al., 2022) however, detailed information on the risk, or hazard of such substances to human and environmental health has not yet been collated. As such, the goal of this systematic evidence map is to identify, organise and map the available evidence on the (eco)toxicological effects of FR substances across ecologically relevant endpoints.

Methods: The methodology set out in this protocol serves to document decisions made *a priori* regarding the conduct of the systematic evidence mapping. We will search several electronic academic (PubMed, Web of Science, Google Scholar) databases, and grey literature sites (OpenGrey) for existing evidence on the (eco)toxicological effect of FR substances to the environment. Eligible studies must contain primary research investigating the risk (or hazard) of one or more FR substances and study an ecologically relevant adverse effect. Ecologically relevant effects include impacts on growth, development, survival, reproduction and behaviour. Taxonomic groups considered for inclusion are those classified as animal, plant, bacteria and/or fungi. Human data will not be included. Articles will be screened in two phases – firstly, Title and Abstract, before a full-text review. A single reviewer will screen all articles with an independent reviewer confirming articles for exclusion. Assessment of each article's quality will not be assessed for this evidence map. Results of the evidence map will be published in a narrative summary and visualised in a publicly available interactive map.

#### **6.07.P-Th532 Implications for Implementing the "Essential-use" Concept in Chemical Regulations**

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The essential-use concept was first introduced in 1987 in the Montreal Protocol to phase out the use of ozone-depleting substances, except for certain essential uses. More recently, the European Commission proposed to use the concept in the decision-making processes of restriction and authorisation under REACH, with the aim to render the processes more efficient and aligned with societal needs. This study aims to inform the ongoing EU work on the criteria for the essential-use concept. It analyses how existing regulatory outcomes from the REACH restriction and the Stockholm Convention differ from the essential-use concept, and the reasons for eventual discrepancies, by determining the main reason justifying granting a derogation from existing restrictions. To do so, it analyses the opinions of the socio-economic analysis committee and POP review committee following the READ approach. The REACH restriction dossier on intentionally added microplastics, and the listing of PFOA under the Stockholm Convention serves as case studies to check whether, by using the same information, an outsider would reach the same conclusion when applying the essential-use concept. An overall evaluation of previous decisions on application for authorisation is also performed to determine number of cases for which the essential-use concept could have been applied to. The results suggest that 100% of the previous exemptions under the Stockholm Convention and 32% of the derogations to restrictions under REACH were recommended for reasons related to the essential-use concept. Furthermore, it has been estimated that the essential-use concept could have influenced the conclusions in 28% of those cases. Regarding the REACH Authorisation, the essential-use concept could have been applied in 95% of the cases. The case study on microplastics demonstrates that enough information is provided in the restriction dossier to perform an essentiality assessment overall, but more information on concrete applications of the microplastics is needed in some cases. Similar observations can be made in the case of PFOA. Overall, this study suggests that it is not needed to drastically change the chemical regulations to implement the essential-use concept in the decision-making, but it would implicate that the industry would need to provide more technical information on the actual functions provided by a substance of concern if they wish to get a derogation for their use.

#### **6.08.A Nano and Advanced Materials Safety: Research Progress, Industrial Applications and Regulation**

##### **6.08.A.T-01 Nano-Adsorbents for sustainable remediation: efficiency evaluation and fate after utilization**

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Nano-adsorbents have emerged as efficient agents for mitigating environmental contamination, particularly in the removal of heavy metal cations and oxy-anions. While their efficacy in pollutant removal is well-established, questions surrounding their disposal and long-term environmental impact require careful consideration. This study delves into the intricate dynamics of

diverse nanocomposites, examining their contaminant removal capacities, redox-transformation capabilities, and fate in simulated landfill conditions. Through a sequential leaching approach, the research sheds light on the nuanced interplay between nano-adsorbents and environmental matrices, emphasizing the importance of post-contaminant removal fate. The findings contribute valuable insights to the development of sustainable cleanup technologies by delineating disposal strategies that prioritize environmental resilience.

Notably, redox-active nanoparticles, characterized by strong contaminant binding, advocate for possible safe disposal practices. Conversely, weak binders like layered double hydroxides offer the potential for contaminant recovery and reuse. This comprehensive understanding of nano-adsorbent behaviors informs the development of environmentally conscious cleanup technologies, striking a balance between efficiency and the mitigation of long-term environmental risks. As we navigate the complexities of nano-adsorbent applications, these insights pave the way for resilient environmental management strategies in the face of escalating pollution challenges.

#### **6.08.A.T-02 Nanofertiliser Use Can Reduce Volatilized N Emissions from Soil – Reducing the Pollution from Crop Production**

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Nanomaterial usage in agriculture offers several possibilities for enhancing a crop's yield, quality, and resilience to stress. A key challenge in current agricultural practice is that excess Nitrogen (fertiliser) application causes water pollution through runoff and leaching and contributes to air pollution through production of the (greenhouse) gas N<sub>2</sub>O and volatile NH<sub>3</sub>. Using nanomaterials in conjunction with synthetic nitrogen fertiliser, may enable more targeted delivery of nutrients to plants. This increase in nutrient uptake efficiency would minimise reactive nitrogen (Nr) losses leading to more sustainable agriculture. This body of work screened a wide variety of engineered nanomaterials for their efficacy in seed priming before taking promising candidates forward for study in soil-based application as nanofertilisers. From the initial screening list of 40 nanomaterials of various composition, a small selection of zeolites and metal-oxide nanomaterials were delivered to lettuce via soil with leachate and volatilized N gases sampled over the 8-week growing period. In addition, after destructive sampling of the lettuces, nanomaterial endpoints of agglomeration, dissolution, and chemical changes to the constituent elements (speciation) were monitored. Initial results indicate that the metal-oxide Ce<sub>0.75</sub>Zr<sub>0.25</sub>O<sub>2</sub> co-application with a half-dose of NPK (nitrogen, phosphorus and potassium) fertiliser was able to decrease volatilisation of N<sub>2</sub>O and NH<sub>3</sub> gases compared to conventional NPK fertilisation, with similar decreases also seen for ammonium, phosphate and nitrate concentrations in the leachate. This work posits that nanomaterial co-application with reduced amounts of synthetic nitrogen fertiliser could be a viable technique in the future of agriculture to maintain yields of lettuce while actively reducing Nr emissions from agricultural soil. Different nanomaterials however have widely differing impacts on different crops (e.g., wheat responded differently), with differences in nutrient use efficiency and agricultural pollution impacted by a range of nanomaterial and soil parameters, indicating that careful nanomaterial design and testing is necessary.

#### **6.08.A.T-03 Integrated Approaches for Environmental Safety Assessment of Nanofoms Embedded in Paint Fragments – SAbyNA Project**

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Implementing nanotechnology in new products is occurring rapidly, contributing to new and improved products in many sectors including energy, health, safety, etc. Ensuring that these innovative nano-enabled products (NEPs) are safe and sustainable for human and environmental health has become a priority for the European Commission and a challenge for industries to comply with the recommended frameworks. In this sense, the aim of SAbyNA project (H2020-NMBP-15-2020) is to generate a digital Guidance Platform to facilitate the development of Safe and Sustainable-by-Design (SSbD) nano-enabled products, encouraging industrial companies to implement the SSbD Framework proposed by the EC. This project has evaluated several case studies from different industrial sectors, including the paint industry, which has included different nanofoms (NFs) in their formulations in recent years. The specific structural characteristics of the NFs such as their size, shape, and greater surface area allow modifying the properties of the newest generation of paints enhancing their quality, durability, performance, etc.

A paint case study in SAbyNA has been the focus of environmental persistence and toxicity evaluation in this study: mixed metal oxide nanoparticles (MMO NP) embedded in polymeric paint matrixes characterized and shipped by CEREGE and ALLIOS collaborators.

This study has addressed the persistence (biodegradation evaluation) and the (eco)toxicological hazard profile of the NEP in the aquatic environment, via the integration of different standardised methods, such as: i) Microbial respiration inhibition: based on the OECD 209 merged with the OECD 301F and the ISO 14851:2019 and ii) algae acute aquatic toxicity assay, OECD 201, studies performed before and after biodegradation. These studies showed that the addition of NFs (MMOs) into

paints leads to an improvement of the (eco)toxicological profile of the paints studied, while also slightly reducing their persistence.

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#### **6.08.A.T-04 Safe and sustainable by design approach for the safety assessment of advanced bio-based nanomaterials used for the production of polyurethane foams**

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Bio-based nanomaterials (B-NMs), derived from biomass and renewable resources, may be applied in the production of polyurethane (PUR) foams used in construction, automotive, and other industries to confer them special properties (e.g. antibacterial, flame retardant). However, the human and environmental safety associated to the exposure to these new advanced materials (AdMa) and resulting nano-enabled products (NEPs), is poorly investigated. Here we report a safe and sustainable by design (SSbD) approach that has been adopted for the safety evaluation of AdMa and NEPs throughout their life cycle. The framework is based on consecutive steps. First, field monitoring campaigns are performed at different pilot lines (PLs) for the production of PUR foams. Aerosol emissions are characterized by using different particle counters and smart sensors. The particle number concentration (PNC) and lung deposited surface area (LDSA) values are measured along the different steps of the production processes. Secondly, the NPs hazard assessment are performed, with *in vitro* models representative of the lung and immune system (co-culture of A549 cells + THP-1-derived macrophages), also cultivated at the air-liquid interface (ALI). Then, key events related to cytotoxicity, oxidative stress and inflammatory responses, according to an adverse outcome pathways (AOPs) oriented strategy, are measured. In parallel, the safety of B-NMs on the environment are evaluated through the fish acute toxicity test (FET) using zebrafish. Finally, leaching test on fresh and artificially aged PUR foams will be performed, characterized and tested by FET to retrieve safety data on the use phase and end-of-life of the new materials. Data from monitoring campaigns showed that the PNC values vary depending on the production processes (e.g. mixing, foaming, cutting) and PL technical characteristics. Results from *in vitro* models, showed that B-NMs are safe considering the endpoints analysed. Also, the B-NMs did not affect the development of the embryos, in terms of mortality and malformation rates. The proposed SSbD framework, through exposure and hazard assessment at the different stages of NPs and NEPs life cycle, contributes to obtain a more realistic interpretation of the risk of innovative AdMa and provides new awareness for defining better decision criteria that will support a more rapid intake of novel safe(r) NEPs in the market. The work was supported by EU-H2020 project BIOMAT, GA n. 953270.

#### **6.08.B Nano and Advanced Materials Safety: Research Progress, Industrial Applications and Regulation**

##### **6.08.B.T-01 Inhalation hazard of multicomponent Advanced Materials: a case of successful confirmation of New Approach Methodologies (NAMs) by *in vivo* data**

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Oxide-based perovskites designed for automotive catalysts contain multiple metal elements whose presence is crucial to achieve the targeted performance. Due to their multicomponent character and their enhanced functionality, they are regarded as Advanced Materials.

They are highly stable in the exhaust operating conditions; however, little is known about their stability in physiological conditions. As some of the metallic components are hazardous to humans and the environment, perovskite's benefits in cleaner air must be balanced with their safety.

Within HARMLESS (GA no. 953183), for the risk screening at earlier design stages, we rely predominantly on Novel Approach Methodologies (NAMs) due to ethical reasons, speed, and parallel testing of several versions. Here we selected *in chemico* Novel Approach Methodologies (NAMs) from the Integrated Approaches toward Testing and Assessment (IATAs) for inhalation: bio dissolution in lung simulant fluids and surface reactivity assay.

We found that the composition and surface properties of six different Lanthanum-based perovskites compromise their stability in the physiological conditions of the lung, influencing the oxidative damage of the particles and the bioaccessibility of leaching metals. At pH 7.5 and 4.5 simulant fluids, both relevant for pulmonary clearance, dissolution depended minimally on the Palladium or Platinum doping but rather on the content of Nickel and Cobalt. We observe consistent biotransformation of the perovskite materials in acidic pH, where the leached Lanthanum ions, but not other metals, re-specified into lanthanum phosphate nanoparticles, increasing the observed oxidative damage in a non-synergistic way.

The *in chemico* results were confirmed by the measured multi-component clearance from lungs into urine after *in vivo* inhalation by male Wistar rats, and were benchmarked against well characterized spinels materials for ranking purposes. Our case study showcases how *in chemico* NAMs are suitable for grouping and read across of multicomponent Advanced Materials, being valuable tools for early stages hazard screening, in a Safe and Sustainable by Design (SSbD) context.

#### **6.08.B.T-02 Acute and Subacute Toxicity of Micro- and Nanoplastics from 3D printing: a Repeated 28-day In Vitro Approach Using an Advanced Human Bronchial Epithelial Model**

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It has been shown that the 3D printing process may release volatile organic compounds (VOCs) and micro- and nanoplastics (MNPs). Once inhaled, its deposition in the respiratory system may induce acute, subacute and chronic adverse effects. As animal models not always provide relevant human toxicity data, New Approach Methodologies (NAMs) based on advanced in vitro models are highly recommended. Among these advanced in vitro models, primary human bronchial epithelial models (PBEC, i.e. MucilAir<sup>®</sup>) are promising models. The objective was to evaluate the potential acute and subacute effects of MNPs release from 3D printing on the respiratory system. We tested the acute and subacute toxicity of polycarbonate (PC) MNPs (< 5 µm) with and without single wall carbon nanotubes (SWCNT) and polypropylene (PP) MNPs (< 5 µm) with and without silver nanoparticles (Ag) on the in vitro PBEC model. PBEC inserts were exposed to 0.1, 10 and 1000 µg/mL of all MNPs for 4h per day up to 28 days. Cytotoxicity, cell barrier integrity and inflammatory response were monitored at time 0 and 4h, and at days 7, 14, 21 and 28. Cell internalisation, genotoxicity, and oxidative damage were measured at day 28. According to the particle size distribution analysis based on number size distribution performed at the TEM, all samples contained few particles > 1 µm (1-13%), and the majority varying from 0.25 to 0.30 µm. EDX analysis showed that in PP+Ag sample, a low percentage of Ag nanoparticles (0.425 wt.%) was detected, whereas PC+SWCNTs sample contained 0.052 wt.% SWCNT. Based on the in vitro results, acute exposures to MNPs (4h) decreased the barrier integrity, being the effects more evident 7 days after of exposure. In the same line, 4h of exposure increased the cytotoxicity, but higher effects were seen 7 days after. Similar toxic potency was observed comparing PC and PP, and no differences were found between PC and PC+SWCNT, whereas PP+Ag was more toxic than PP alone. Preliminary results indicate that repeated short-term exposures (4h) to MNPs with and without nanomaterials induce acute but mainly subacute effects (up to 7 days). Therefore, PBEC is a sensitive in vitro tool able to discern differences in particle toxicity in the presence and absence of nanomaterials, thus being useful for inhalation risk assessment of particulate materials. This work was supported by the EU H2020 Project SAbyNA (GA no. 862419).

#### **6.08.B.T-03 Case studies assessing suitability of in silico modelling tools and read-across approaches for nanomaterial hazard assessment**

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The study presented here gives insight on available frameworks and state-of-the-art developments for read-across and other *in silico* approaches, which can be alternatives to conventional nanomaterials (NMs) hazard and risk assessments. During this study a systematic literature review was carried out to identify the available models and tools for the computational hazard and risk assessment of NMs.

This review was followed by an analysis to map the aspects of models that are fully implemented (model development, validation, and documentation), as well as identifying potential data gaps and flaws which could be improved. Next, expert opinions were collected and incorporated in the findings to present a complete and integrated view of the nanoinformatics field. Finally, the applicability, the strengths and limitations of the models were demonstrated through the development of three case studies using as many of the identified tools as possible.

Based on the collected results, we conclude that the nanoinformatics field is moving forward, as there are already available reliable models and the infrastructure needed to implement them as user-friendly tools (e.g., supported by different EU-funded projects). Nonetheless, there is room for improvement regarding the inclusion of information on the applicability domain of the models within the tools and the availability of training material for each tool as illustrated by the case studies. Confidence of the relevant stakeholders in the developed models is still low, mainly due to the limited modelling information from the developers, the lack of sufficient data to provide the correct applicability domain, and the lack of validation with conventional experimental methods. Building trust in the use of appropriately reported and validated computational methods that are also

scientifically supported, is therefore the next step towards *in silico* approaches gaining widespread public and regulatory acceptance as alternatives to conventional hazard and risk assessments.

#### **6.08.B.T-04 Implementation of Grouping Strategies for Nanoecotoxicology**

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Nanoecotoxicology focuses on the hypothesis that alterations to the nanoform can influence ecotoxicological potential. It has also been hypothesized the pristine chemical properties can be used to predict ecotoxicity. Grouping and read-across approaches have been proposed to streamline regulatory compliance, as assessing novel products on a case-by-case basis is not effective governance. Predicting risks for novel materials, however, requires access to historical data.

The aim of this work is to highlight lessons learned from the NanoReg2 project and to emphasize the need for FAIRification of nanomaterial research to successfully implement grouping approaches. The research focuses on representative industrial nanomaterials available at the European Commission's Joint Research Centre's Nano Repository and nanomaterials from industrial partners associated with the NanoReg2 project. Fish cell lines, mussel hemocytes, algae, and *Daphnia magna* were all exposed to the same nanomaterials, whose suspensions were prepared using the same protocol, to establish a comprehensive dataset with comparability among nanomaterials.

Modelling through multiple correspondence analysis (MCA) assessed the relationship between pristine physicochemical properties, system dependent physicochemical properties and ecotoxicological responses. The presented work establishes one of the most robust data sets for analyzing the relationship between physicochemical properties and ecotoxicological endpoints. The model focused on two key questions: 1) the relationship between biological endpoints and the description of ecotoxicity and, 2) how key physicochemical properties describe ecotoxicity. Results are preliminary; however, the MCA model demonstrated that there are clear separations of data based on the ecotoxicological test conducted.

This research is a representative first step in exploring the hypothesis that pristine physicochemical properties can be used in read-across approaches to predict ecotoxicity of untested nanomaterials with similar physicochemical properties. Access to high quality data with well characterized properties is crucial for the development of predictive modelling. One of the major limitations in the analysis is that system dependent properties of nanomaterials play a major role in the discrimination between nanomaterials. This challenges regulatory paradigms as testing environment will lead to a different behavioral profiles.

#### **6.08.P Nano and Advanced Materials Safety: Research Progress, Industrial Applications and Regulation**

##### **6.08.P-Th533 Assessment of the Advanced Material Molybdenum Disulfide (MoS<sub>2</sub>) and Layered Double Hydroxides (LDHs) Nanosheets Effects *in vitro* Using Zebrafish Liver Cells**

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Among the emerging advanced materials, layered nanostructures such as molybdenum disulfide (MoS<sub>2</sub>) and layered double hydroxide (LDHs) nanosheets are increasingly being applied in several fields including environmental remediation. During the production, use and disposal, these material will reach the environment. However, their potential hazard is poorly understood. Due to the continuous development of novel nanomaterials (NM), their diversity in composition and structure and subsequent needs for hazard assessment, effective strategies to assess their environmental impacts are needed. The use of *in vitro* models can contribute to increased understanding of molecular and cellular mechanisms in a high-throughput and cost-effective manner and can address the challenge of the plethora of NM continuously being produced. Fish cell lines can be versatile tools as non animal methods (NAMs) to assess the effects of NM, among other contaminants, in the aquatic environment. The aim of the study was to elucidate the behaviour, potential biocompatibility and effects of the novel layered nanostructures MoS<sub>2</sub> and Mg-Al-LDH *in vitro* using the zebrafish liver cell line ZFL and evaluate its suitability in nanotoxicology studies.

In the first set of experiments, the cells were exposed to increasing concentrations of MoS<sub>2</sub> and LDH for 24-48h. The effects on metabolic activity, membrane integrity, lysosomal integrity and reactive oxygen species (ROS) formation were assessed. NM interference controls were included to assess the potential NM interference with the respective assays. The NM in the stock dispersions and exposure media were characterised with transmission electron microscopy (TEM) and dynamic light scattering (DLS). The first results show no adverse effects of the LDH NM *in vitro* despite the high exposure concentrations used which presents their potential as a novel material for environmental applications. A decrease in metabolic activity and membrane integrity was observed upon exposure to MoS<sub>2</sub> nanosheets, albeit at high concentrations. The formation of ROS appeared to be the most sensitive endpoint in the ZFL cell line. Ongoing work focuses on gene expression and NM uptake studies *in vitro* as well as comparing *in vitro* with *in vivo* responses using the zebrafish embryo test.

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#### **6.08.P-Th534 Development of a Human Hazard Strategy for Assessment of Nanoforms and Nano-Enabled Products in the SAByNA Guidance Platform**

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The SAByNA Guidance platform is an integrative and interactive web-based service that gives the industry support towards the development of safer nanoforms (NFs) and nano-enabled products (NEPs) over their whole life cycle, considering hazard, exposure, functionality, Life Cycle Assessment, and costs. The hazard assessment is a critical part of the platform which aim is to identify potential hazards of a NF or NEP at an early stage of product development, preventing the user from large investments in money or time and guiding them towards Safe(r) by Design interventions.

The human hazard strategy developed within the platform starts with a screening phase requiring information on chemical composition, physicochemical properties, and likely exposure routes. CLP classification of the NFs is considered, and preventive flags are raised if Substances of Very High Concern or harmful substances are detected. For a more thorough assessment, the user is guided towards a hazard strategy tailored for inhalation and dermal exposure, considering several toxicity endpoints in a hierarchical manner: dissolution rate in physiological fluids, cytotoxicity, genotoxicity, reactive oxygen species generation, and inflammation. Additional endpoints as skin irritation and absorption, and barrier integrity are considered for dermal exposure. Specific guidelines on data retrieval from databases and on data generation using an in vitro testing strategy are provided. Toxicity tests were selected and optimised for their efficiency in use and applicability to NFs considering relevant use conditions and end-of-life. Meaningful thresholds of effects were set up and the results for the different endpoints were combined for final outputs on the NFs potential hazard. The applicability of the strategy was verified in two case studies for two different industrial sectors, paints and 3D printing. To validate its predictive capacity, the outputs of the hazard strategy were compared to existing in vivo hazard data or data obtained using higher tier toxicity testing.

The resulting hazard assessment strategy provides the industry with useful guidelines and tools on data gathering and a set of easy-to-use, robust and cost-effective testing approaches for the prediction of potential NFs hazards. This early warning system supports the implementation of safe-and-sustainable-by-design on NFs and NEPs and is compatible with early stages of product development and along the whole material's life cycle.

#### **6.08.P-Th535 20 years of European nanomaterial legislation – closing the final gaps**

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Developments within nanotechnology have increased rapidly the past decades. In 2004 two highly influential reports shed, as some of the first, light on the potential negative societal implications of nanotechnology. The reports were published by the Royal Society & the Royal Academy of Engineering and the European Commission, respectively. Both reports provided a list of recommendations on how to address the risks of nanomaterials and the European Commission introduced the so-called “incremental approach” – establishing that existing legislations should be revised, where needed, to cover nanomaterials. In this study we sat out to investigate how the recommendations from the two reports from 2004 have been addressed, now almost 20 years after they were presented, and how legislations have been amended to account for nanomaterials. Furthermore, we map out remaining challenges related to nanomaterial legislation and future steps to overcome these. In order to do this, we analyzed essential European legislations relevant to nanomaterials in accordance to e.g. definitions, information requirements, decision support tools, risk management and strengths and weaknesses. Status on each recommendation in the two reports from 2004 was assessed as met to either a high, medium or low degree. Status on the recommendations were evaluated via targeted literature searches at Web of Science, screening of legislations/test guidelines/guidance documents and expert judgement. We found that 13 out of 29 recommendations have been met to a high degree, 14 out of 29 have been met to a medium degree and 2 out of 29 have been met to a low degree. These findings indicate great achievements within nanosafety and nanomaterial legislation. Nevertheless, as some concerns are still left partly or fully unsolved, further efforts are needed. In particular, future efforts should be prioritised to ensure use of a uniform nanomaterial definition, further development of standardised risk assessment and characterization methods for nanomaterials and further attempts to eliminate/minimise unintentional nanomaterial emissions. In addition, we recommend to strengthen enforcement and implementation of existing nano-specific requirements, as well as revising legislations that do not consider nanomaterials, where needed.

#### **6.08.P-Th536 Ecotoxicity assessment of “smart” coatings for marine corrosion protection**

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Corrosion is one of the greatest challenges facing modern society. It affects a wide range of sectors, from construction to energy production, resulting in substantial financial burdens, safety risks, and environmental impacts. Protective coatings are the most used solution for preventing corrosion; however, most effective anti-corrosion methods lack environmental friendliness. Industry is channeling efforts to find efficient, effective, and eco-friendly solutions to meet the UN Sustainable Development Goals. Nanotechnology plays a pivotal role in finding sustainable approaches to combat corrosion. The immobilization of active ingredients in engineered nanomaterials (ENMs) such as layered double hydroxides (LDHs) or silica mesoporous nanocapsules (SiNC) are promising solutions as they prevent the direct interaction of active compounds with other coating components and allow their controlled release under specific conditions (e.g., presence of chlorides), reducing application rates and environmental releases. Despite LDHs/SiNC being deemed eco-friendly ENMs, their ecotoxicity must be evaluated to ensure environmental safety. The aim of this study was to evaluate the ecotoxicity of “smart” anti-corrosion marine coatings to infer their eco-friendly potential. Coatings with different additives incorporated were tested: zinc aluminium (ZnAl) LDHs stabilised with nitrite, ZnAl LDHs with hexacyanoferrate, ZnAl LDHs with vanadate, and SiNC with phenolphthalein. The experimental design consisted of microscope slides with applied coatings and placed in vials containing artificial seawater. One set of vials had slides with induced damage, another set had intact slides. The test lasted 28 days, and water was collected at different times for chemical analysis and ecotoxicity testing with three marine species: *Phaeodactylum tricornutum* (diatom), *Tetraselmis chuii* (green microalgae) and *Brachionus plicatilis* (rotifer), according to standard test guidelines. Results revealed very low to no toxicity of all coatings to the species, with *T. chuii* being the most sensitive one, while no toxicity was observed in *P. tricornutum*. These results demonstrate the eco-friendly potential of these “smart” coatings and support the environmentally safe application of tested functional nanomaterials in marine anti-corrosion paints. This work highlights the advantages of collaboration between academia and industry for effective implementations of Safe and Sustainable by Design strategies.

#### **6.08.P-Th537 Safe and Sustainable by Design (SSbD) strategies for advanced manufactured materials: a case study on perovskites for catalysis**

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In the EU funded project HARMLESS (GA no. 953183), we develop Safe and Sustainable by Design (SSbD) strategies with focus on nano-enabled Advanced manufactured Materials (AdMa); we aim to guide companies in making decisions during AdMa design stages, when options are many and budget is low.

Previous experience and stakeholder analysis is used to integrate existing and newly generated data & models into a user-friendly Decision Support System (DSS). In this platform, we use predictive tools, non-animal testing (i.e., Novel Approach Methodologies (NAMs)) guided by Integrated Approaches toward Testing and Assessment (IATAs) and established OECD guideline protocols, for assessing safety and sustainability aspects at each innovation stage, named ideation, lab phase and pilot phase.

We tested the DSS infrastructure for six SSbD versions of perovskite materials, component in three-way catalysts, located at the lab phase of the innovation stages. These materials incorporate different amounts of Lanthanum, Cobalt, and Nickel with doping amount of Platinum and Palladium to enhance their performance as exhaust cleaning catalyst, providing cleaner air.

The DSS interface provides specific advice at each innovation stage, addressing human safety aspects (occupational and for consumers), environmental safety, sustainability during the life cycle and AdMa performance. Safety comprises both hazard and exposure aspects. The proposed interface aligns with the latest SSbD draft framework from the Joint Research Centre (JRC) of the European Commission. However, our assessment is not stepwise but remains flexible in order to be more generally applicable to nano-enabled materials that do not consist or contain of particles. The DSS provides advice and recommendations to the user, to find the best balance of performance, safety, and sustainability.

Our study shows that among the six SSbD versions of the perovskites in analysis, the one with the best compromise between all aspects, is the one consisting of 8% in weight of Nickel and Palladium doping (LaCoNi\_Pd).

#### **6.08.P-Th538 Multispecies ecotoxicity assessment for “safe-by-design” of metal nanofoms for paints – a case study of SAbYNA project**

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The “safe by design” (SbD) approach is a proactive strategy used in various fields, including the paint industry, to integrate safety considerations into the design and development of products from the outset. In the context of paints, adopting an SbD

approach involves considering the potential hazards associated with the components and manufacturing processes, and taking steps to minimize or eliminate these hazards at the earliest stages of product development.

The SAbYNA project (H2020-NMBP-15-2020; <https://www.sabyna.eu/>) was developed with the main aim to produce a Guidance Platform for the industries to eventually implement safe-by-design more efficiently and cost-effectively. Within this project, several case studies from different industrial sectors have been evaluated, including the paint industry, which has included different nanoforms (NFs) in their formulations in recent years. The specific structural characteristics of the NFs such as their size, shape, and greater surface area allow modifying the properties of the newest generation of paints (NEPs: nano-enabled products) enhancing their quality, durability, functionalization, etc. However, SbD must also consider safety aspects related to the environmental compartment at the innovation stage to support decision-making. Zinc-based mixed metal oxide (MMO) nanoparticles (size 1.85 – 7.06 nm) were found to have more efficient functionality for the formulation of outdoor paintings. Three Zn/Al-based nano-MMO were synthesized at different annealing temperatures (1000, 800, and 600°C) and coated with an organic ligand (\_C). Previously performed functionality assessments, selected coated nano-MMO synthesized at 1000°C (nano-MMO 1000\_C) as most efficient for solar reflectance. To test which NF could be the safest alternative for the environment, an ecotoxicity screening was performed using *Daphnia magna* and microalgae *Raphidocelis subcapitata* toxicity assays. Results showed the micro algae to be more sensitive than daphnia. Nano-MMO 1000 with and without coating presented similar toxicity, therefore, the organic coating did not affect the toxicity of nano-MMO 1000. The remaining NFs were not found toxic. In addition, algae growth inhibition was found correlated to persistent prevalence of large aggregates of nano particles (~300 to 460 nm) in the exposure medium.

#### **6.08.P-Th539 Ecotoxicological Assessment of Graphene Containing Commercial Product in Aquatic Ecosystems: From Single Species Approaches to Aquatic Microcosms.**

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Graphene-based nanomaterials (GBMs) are garnering increasing global attention due to their unique properties. However, this rapid expansion raises concerns about potential environmental risks associated with their environmental dissemination occurring throughout their life cycle. Although ecotoxicological studies have been conducted on raw materials, commercial products are overlooked yet. The objective of this research is to assess the toxicity towards aquatic ecosystems of a commercial ink composed of few-layer graphene (FLG), dispersed with sodium deoxycholate (SDC) as dispersant. Standardized single-species tests were conducted on organisms such as algae (*R. subcapitata*), crustaceans (*D. magna*) and amphibians (*X. laevis*). Additionally, experiments were carried out using microcosms. The systems were composed of natural biofilms and chironomids (*C. riparius*) as primary consumers. The metabolic state and diversity of biofilms were monitored, along with the growth and emergence dynamics of chironomids. At environmentally relevant concentrations (0.1 mg/L), both the commercial formulation and SDC alone induced disturbances in microbial communities, suggesting a potential imbalance in the ecological functions performed by these taxa. Furthermore, a decrease in chironomid growth was observed at the larval stage for the commercial product and dispersant, while the effects were maintained in adults only exposed to SDC alone. Disturbances were also noted in sex ratios at emergence, thereby possibly threatening the species' life history traits and survival under natural conditions. Overall, the results from both single-species tests and microcosms underscore a strong effect of the dispersant on the toxicity of the evaluated product, with a mitigation of SDC toxic potential through its interaction with FLG. This study provides a realistic assessment of FLG toxicity through a commercial formulation under environmental conditions.

Keywords: Nanomaterials, Aquatic ecotoxicology, Microcosm

#### **6.08.P-Th540 Regulatory testing of fate (OECD 29, 318) and aquatic toxicity (OECD 201, 211) on selected ZnO nanoforms**

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In 2019, the REACH-registration dossier of ZnO underwent an evaluation by the European Chemicals Agency (ECHA) who decided on the need for data on fate and effect for all nanoforms present on the EU market. Until then, the registration dossier listed a large number of ecotoxicity studies, including fish, aquatic invertebrates, algae and aquatic plants. Based on these literature data it was determined that nanoforms of zinc oxide are less or equally toxic than the dissolved Zn<sup>2+</sup> cations (i.e., the hypothesis being that zinc ions represent worst-case toxicity). However, all of the tested ZnO nanoforms covered by the literature featured no coating and most targeted primary particles sizes around 30 nm, meaning not all of the Zn nanoforms available in the EU market were covered. Furthermore, there were also a few studies in the dossier that contradicted the ion-worst-case-toxicity hypothesis. Therefore, the relative toxicity and fate of all available nanoforms remained unclear from ECHA's perspective.

A testing scheme was then specified in the evaluation decision to determine the specific effect and fate of the zinc oxide nanoforms. This testing scheme was divided into two steps. In the first step fate data regarding the particle



transformation/dissolution (TD; OECD TG 29) and dispersion stability (OECD TG 318) was requested for 28 zinc oxide nanoforms, which represented the nanoforms available in the EU market at this time. The fate of the 28 nanoforms was screened to classify the particles in terms of their solubility and stability. The testing scheme stated that nanoforms should be grouped according to: the highest, lowest and a mean dissolved Zn<sup>2+</sup> concentration (based on the results from the TD test, OECD TG 29); and to low dispersion stability, high dispersion stability and condition-depending dispersion stability (based on the results from the dispersion stability testing, OECD TG 318). In the second step, representative nanoforms from the aforementioned groups were chosen to investigate their chronic toxicity to aquatic organisms using the freshwater algal growth inhibition test (OECD TG 201) and *Daphnia magna* reproduction test (OECD TG 211) considering nano-specific test adaptations as available at the time, when the OECD GD 317 was not available yet.

The poster will present the outcomes of the fate and aquatic toxicity studies, the lessons learned from this process and recommendations for improvement of testing nanomaterials.

#### **6.08.P-Th541 Ecotoxicity assessment of ENMs: harmonization of *Daphnia* standardized protocols.**

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Proper characterization and safety assessment of engineered nanomaterials (ENMs) face major challenges due to their unique traits (e.g., small size, high surface area, potential for reactivity), making it difficult to assess and foresee the ecotoxicity of ENMs in biological systems.

Appropriate Test Guidelines (TGs) and Guidance Documents (GDs) are necessary to ensure that requirements are met for evaluating and approving ENMs, for example, in European regulations like REACH. TGs and GDs are crucial in establishing standardised dispersion procedures and ensuring compliance with conditions for assessing ENMs toxicity. Standardised dispersion methods address issues unique to unstable ENMs, such as preventing/reducing agglomeration/aggregation to improve material dispersion throughout testing. These standard approaches will also produce more consistent and reliable data across investigations. This work is within the scope of the NanoHarmony project, which supports the development of TGs and GDs for test methods tailored to ENMs deemed a regulatory priority. Challenges include accounting for water column ENMs' behaviour (e.g., sedimentation and agglomeration) and various exposure methods, such as test vials of different materials or dimensions, which may result in different behaviours, exposures, and toxicity.

Taking this into consideration, the aim of this study was to 1) evaluate the efficiency of the dispersion of ZnO NM110, MWCNT NM400, Bentonite NM600, and TiO<sub>2</sub> NM104 ENMs according to the Enhanced Dispersion Method of The NanoReg protocol, 2) evaluate the ecotoxicity of these ENMs to *Daphnia magna*, following the Acute Immobilisation Test (OECD No. 202). For this, various exposure systems were used to compare the effects of using plastic vs. glass setups and test vials with different depths (petri dish vs. flask).

Overall, results demonstrated higher toxicity of *D. magna* exposed to ZnO NMs (LC50 from 1.63 mg/L to 2.36 mg/L), while no toxicity was found for Bentonite NMs (LC50 > 500 mg/L). Lower LC50 values were obtained in the plastic experimental setups compared to the glass one and with a reduced water column. This study provides valuable information and recommendations to aid in adapting standardising techniques and methodologies, such as the Acute Immobilisation Test of OECD No. 202, for assessing the hazard of ENMs in *D. magna*.

#### **6.08.P-Th542 Developmental Effects of Cadmium Telluride Quantum Dot Nanoparticles in Zebrafish Embryo and Larva**

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Manufactured nanomaterials (MN) which have applications in biomedical devices, agriculture, and many industries pose serious concerns to the environment and human health. Cadmium-based materials are known to cause structural and functional alterations in fish, and are even highly toxic during embryonic and early larval developmental phases at low concentrations. Cadmium telluride quantum dot nanoparticles (CdTe QDs) are used in the manufacture of semiconductors that are being used in biomedical applications. Therefore, in the present investigation to understand the developmental effects, zebrafish embryos after fertilization were exposed to environmentally concerned CdTe QDs at the concentrations 0.156, 0.313, 0.625, 1.25, 2.5 and 5.0 mg/L and observed up to 216-hours post fertilization (hpf).

The CdTe QDs used were water-dispersible, highly stable, 3-mercapto propionic acid-functionalized having uniform spherical form with an average size of 3.8 ± 0.2 nm. Each group had 20 zebrafish embryos exposed individually in a 24-well plate

having solutions prepared by directly mixing the CdTe QDs in water.

The study showed that complete hatching of eggs happened between 48 and 72 hpf and the hatching rate at control was 83% whereas CdTe QDs groups exhibited 55 to 75%.

Varying coagulated embryos were observed at 24 hpf across the groups including the control. No toxic signs and mortality of larva were observed in control and CdTe QDs groups of 0.156 and 0.313 mg/L. The first mortality of larva was observed at 5.0 mg/L at 72 hpf and the percent mortality at 216 hpf was 27.3, 30.8 and 64.9 % of hatched larvae at 1.25, 2.5, and 5.0 mg/L, respectively. Toxic signs first appeared around 96 hpf with scoliosis at 2.5 mg/L, and scoliosis and lordosis at 5.0 mg/L; overall toxic signs at 216 hpf were observed at 0.625, 1.25, 2.5, and 5.0 mg/L.

In conclusion, CdTe QD nanoparticles were found toxic to zebrafish embryos even at 0.156 mg/L at 24 hpf, causes detrimental effects to hatched larvae at 0.625 mg/L appearing at 168 hpf but with no mortality till 216 hpf and induces toxic signs and mortality at 1.25, 2.5 and 5.0 mg/L under tested conditions.

#### **6.08.P-Th543 Assessing Pre-guideline Literature Data on Bioaccumulation of CeO<sub>2</sub> Nanoparticles in a REACH Context – Conclusions, Identified Issues, and Recommendations for Standardised Testing**

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In view of the update of the REACH dossier for CeO<sub>2</sub>, the available literature on bioaccumulation of CeO<sub>2</sub> nanoparticles in aquatic and terrestrial organisms was evaluated. In these studies, the uptake, bioconcentration, bioaccumulation, and trophic transfer was typically assessed based on measurements of cerium in the organisms and/or the exposure medium. These studies indicate poor bioconcentration/bioaccumulation in both aquatic and terrestrial organisms, as well as trophic dilution. Although there are currently no standard testing guidelines or recommendations for investigating bioaccumulation of nanomaterials, it is obvious that the uptake and bioaccumulation of the CeO<sub>2</sub> nanoparticles as such should be investigated as well next to the total, Ce-based uptake and bioaccumulation. The few studies that addressed this in their experimental setup used various techniques to identify nanoparticles in the organisms studied. However, in most studies, no quantification is done and therefore no bioaccumulation factors are derived or can be derived from the observations. In this poster, we will inform on the conclusions that could be drawn for the aquatic and terrestrial bioaccumulation endpoints based on the available non-standard studies, as well as the implications for potential exposure assessment. We will also discuss the need for standardised testing, to be able to differentiate between bioaccumulation of nanoparticles with different properties and to understand how environmental conditions may affect bioaccumulation of nanoparticles.

#### **6.08.P-Th544 Towards Improved Reliability in The Characterization of Metallic Nanoparticles in Environmental Samples**

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The use of metallic nanoparticles (NPs) has exponentially grown in recent years owing to their exceptional physical and chemical attributes at nanoscale. This widespread application inevitably leads to their growing discharge into the environment, but limited knowledge exists regarding NP fate and the possible alterations that they might undergo upon release. Thus, the characterization of NPs within environmental samples is crucial for understanding their behaviour, potential risks, and impact on ecosystems and human health. The quest for enhanced reliability in this characterization process is paramount, considering the inherent NP nature and their ubiquitous presence in various environmental matrices.

The complete NP characterization involves not only the determination of size, shape, or composition typically obtained by microscopic techniques, among others, but also quantitative information about NP (mass and number) concentrations. However, ensuring the reliability and accuracy of these measurements remains an analytical challenge due to the complexity of environmental matrices which results in limitations in sample preparation and/or the analysis. Consequently, there is still the need to develop innovative analytical methods capable of reliably detecting, characterizing, and quantifying NPs in relevant environmental and toxicological systems.

In this context, inductively coupled plasma mass spectrometry in its single particle mode (SP-ICP-MS) has emerged as an interesting alternative to conduct a simultaneous sizing and counting of NPs as well as the quantification of its ionic forms. The potential of this approach for the NP characterization in complex samples may be hampered by matrix effects affecting the adequate discrimination between ionic and particulate signals.

To overcome this limitation and improve the performance in challenging scenarios, in this work, an analytical tool of high metrological quality such as the isotope dilution analysis (IDA) has been explored in combination with SP-ICP-MS. The potential of IDA to better discern between signals has been thoroughly evaluated using platinum NPs as a model. Limitations related to matrix effects caused by different environmental factors have also been assessed. Our findings suggest that IDA-SP-ICP-MS holds promise as a tool for characterizing metallic NPs in environmental samples. Nevertheless, further comprehensive investigations are necessary to fully understand its capabilities.

#### **6.08.P-Th545 Hydrodechlorination of Mine Water-specific Polychlorinated Biphenyls (PCBs) Using Palladium Nanocatalysts**

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Polychlorinated biphenyls (PCBs) were used, amongst others, in mining for fire protection reasons until they were banned worldwide. Even today, mines can still be contaminated with PCBs from leaks and abandoned equipment [1]. Mine water must be continuously pumped out and discharged into surface waters. Unlike for diffuse sources, efficient technologies could be easier applied to these point sources to reduce environmental impact.

A perspective promising approach is the catalytic dechlorination of PCBs using palladium (Pd) nanoparticles. The aim of the present study was to determine the dechlorination rates of selected mine water specific PCBs both under optimal reaction conditions and in matrix-rich mine water. For dechlorination experiments, small amounts (150 µg L<sup>-1</sup>) of suspended Pd nanoparticles were added to both PCB containing laboratory solutions and mine water. The experiments were conducted under anoxic conditions with hydrogen for hydrodechlorination reactions. Simultaneous extraction and detection of reactants, intermediates and fully dechlorinated biphenyl were performed by SPME-GC-MS [2]. The Pd particles showed high catalytic activities for dechlorination of mine water specific PCBs under optimal conditions (up to 4400 L min<sup>-1</sup> g<sup>-1</sup>), but not in the matrix-rich mine water. Here, the catalytic activity was strongly inhibited by catalyst poisons present in the mine water (e.g. sulfur compounds). Therefore, the consecutive goal is to protect the nanoparticulate Pd catalysts from deactivation by catalyst poisoning. To maintain high catalytic activity, experiments are underway to incorporate the Pd particles into a surface coating of the reaction vessels. Coatings must allow PCB diffusion to the catalysts while at the same time hinder entering of catalyst poison. Beside increased degradation rates, the coating is intended to prevent Pd discharge into the environment during on-site applications, thereby avoiding costs and environmental harm by nanoparticles. To maintain catalyst activity, it is planned to apply an additional protective layer to further protect the Pd particles from catalyst poisons and ensure a long catalyst life. This would allow for in-situ dechlorination of PCBs, making the technology scalable for on-site treatment of contaminated water.

[1] Wiltshcka et al. (2023). <https://doi.org/10.1021/acsestwater.3c00179>.

[2] Wiltshcka et al. (2020). <https://doi.org/10.1016/j.apcatb.2020.119100>.

#### **6.08.P-Th546 Trimethyl chitosan magnetic nanosorbents efficiency and safety - Remediation of waters contaminated with glyphosate and aminomethylphosphonic acid**

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Glyphosate (GLY) stands as one of the most commonly used herbicide worldwide, with its main metabolite being aminomethylphosphonic acid (AMPA). The pressing need for their effective removal from water sources arises from their recurrent presence in both ground and surface waters and the recognized environmental risks they pose. In this study, magnetic nanosorbents, developed by us, comprising magnetite nanoparticle core coated with trimethyl chitosan-silica hybrid shells, were investigated for the adsorptive removal of GLY and AMPA from ultra-pure water and wastewater samples. Additionally, a Fish Embryo Acute Toxicity test with *Danio rerio* was conducted to evaluate the safety of the magnetic nanosorbents, assessing parameters such as survival, hatching, heartbeat rate and malformations occurrence in a 96 hours exposure. The results showed that, in ultra-pure water, the magnetic nanosorbents (0.5 g/L) removed 96% of GLY (initial concentration = 3 µg/L; pH = 5), while for AMPA, a removal of 67% was observed under the same conditions. Notably, increasing the dosage of magnetic nanosorbents to 2.5 g/L resulted in an 89% removal of AMPA (initial concentration = 3 µg/L; pH = 5) from ultra-pure water. These findings indicated that, in ultra-pure water, the magnetic nanosorbents were able to reduce realistic environmental concentrations of GLY and AMPA to levels that are below the maximum permissible value (0.1 µg/L) as defined by the European Directive of Drinking Water. When applied to spiked wastewater samples (initial concentration = 3 µg/L; pH = 7.5), the magnetic nanosorbents (2.5 g/L) removed 42% of GLY and 54% of AMPA, demonstrating the preliminary applicability of these nanomaterials in more realistic conditions. Furthermore, no toxic effects were found on embryos exposed to 2.5 mg/L of magnetic nanosorbents, indicating that these nanomaterials seem safe for zebrafish embryos considering the individual endpoints assessed. Our results collectively suggest that assisted magnetic water remediation using these nanosorbents could offer an efficient and safe process of eliminating GLY and AMPA from contaminated waters. Our study provides valuable insights into the potential for advanced nanomaterials to address the pressing issue of herbicide contamination in aquatic environments.

#### **6.08.P-Th547 Nanoherbicides and its impacts on weed management: an alternative for sustainable agriculture**

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Herbicides have negative environmental impacts but they are still necessary to keep crop productivity and ensure efficient weed control in agricultural areas. Nanotechnology has become an essential tool in improve herbicides effectiveness, increasing toxicity to the target species, leading to effective management with reduced rates of active ingredient (a.i.) due to changes in herbicide delivery. An in-depth investigation concerning the effect of nanoparticles (NPs) in herbicide's mode of action (MoA) in target species is important to a safe and sustained by-design (SSbD) approach. The objective of this work was to highlight the effect of NPs as carriers on herbicide MoA in plants integrating results from radiometric and microscopic

techniques. A critical investigation was done in recent literature using nanotechnology, pesticides, radiometric, and microscopic as keywords in the Web of Science database. In general, the studies with plants use NPs as models, and the effective tracking of pesticide-loaded NPs is not deeply investigated. For example, radiometric techniques (RdT) allowed an evaluation of  $^{14}\text{C}$ -graphene NP translocation through a symplastic pathway in wheat, suggesting that graphene is a nanocarrier through plasmodesmata. In another and more realistic approach, RdT was used to identify the behavior of  $^{14}\text{C}$ -nanoatrazine in plants and  $^{14}\text{C}$ -nanometribuzin in soil. These works highlight herbicides radiolabeled with  $^{14}\text{C}$  as a useful tool for regulatory and SSbD approaches in developing NPs as herbicide carriers. Besides that, complementary techniques as confocal microscopic (CfM) with fluorescent marker is essential for understanding NPs behavior in plants, because radiometric techniques are restricted to mapping the a.i. behavior. CfM was able to study the movement of atrazine-polymeric NPs via root; and was also able to identify stomata as an absorption pathway of nanoatrazine applied through the leaf. Moreover, tracking nanoherbicides needs an interdisciplinary approach to determine the integrated behavior of pesticides and NPs in plants, as well as the effect of NPs in herbicide MoA. This work highlights the importance of understanding the interaction between nanoparticles and herbicides to optimize their effectiveness and minimize potential negative environmental impacts. Additionally, this research opens new possibilities for developing more sustainable and efficient weed control methods in agriculture.

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#### **6.08.P-Th548 Pesticide Nanoformulation Is a Solution for Agriculture? Fate, Efficacy, and Challenges of Metribuzin Herbicide as Case of Study**

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Nanoformulation is reported as a solution for pesticides in agriculture to increase target delivery, protect active ingredients against biotic and abiotic losses, and reduce environmental risks without efficacy decrease. For herbicides, the main pesticide class used in the world, nanoformulations can be a strategy to reduce environmental concentration and manage difficult-to-control weed species simultaneously, mainly for pre-emergent actives that are applied directly in the soil. Metribuzin polymeric nanoformulation was used as a study model for nanopesticide environmental fate and efficacy. Poly- $\epsilon$ -caprolactone (PCL) nanoparticles (NP) were developed based on the nanoprecipitation method, and metribuzin (MTZ) herbicide radiolabeled was encapsulated as a tracer. A radiometric technique was applied to track nanoparticles in soil (mobility, degradation, and non-target effect assays), at field metribuzin dose (480 g a.i. ha<sup>-1</sup>). Also, weed control assays were carried out, with non-radiolabeled nanoparticles to investigate the enhanced activity of metribuzin in broad-leaf plants (0, 48, and 480 g a.i. ha<sup>-1</sup>). Stable nanoformulation for MTZ was obtained with PCL, resulting in 289 nm of hydrodynamic size and -31 mV of surface charge ( $\zeta$  potential) over 120 days. In the soil, nanoparticles showed no increment in the persistence of MTZ herbicide (half-life of 17 d), with similar mobility to the commercial metribuzin treatment (retention factor of 0.45-0.5). The bioaccumulation of MTZ in earthworms was higher (1.76 times) than NP with MTZ. In plants, a different pattern of absorption by roots was observed, concentrated in the vascular system for NP and all-plant distributed for commercial MTZ (3.9-5.4%). MTZ (NP and commercial) reach a satisfactory weed control of *Amaranthus viridis*, even in low doses for MTZ NP. Therefore, MTZ NPs were environmentally safe without compromised efficacy. Our research evidenced that the radiometric technique was an excellent tool for tracking nanoformulations in soil and organisms (non-target and target plants). MTZ nanoformulation can be a relevant tool for weed management in agriculture. Also, other nanopesticides can be developed in the same way. However, a simple solution is not available, and the design needs to be thought out for each specific case of agrochemical, target, and environment to be inserted.

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#### **6.08.P-Th549 Freshwater ecotoxicity and life cycle risks of nano-encapsulated imidacloprid compared to its conventional analog**

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The application of nanotechnologies in agriculture raises concerns about the safety of nanopesticides, yet research has not fully characterized their potential environmental risks compared to conventional pesticides. To support sustainable food production, we compared the ecotoxicity of nano-encapsulated imidacloprid (nano-IMI) and its active ingredient in non-target freshwater invertebrate and embryonic vertebrate models (*Daphnia magna*, *Chironomus kiinensis*, and *Danio rerio*). Results showed that nano-IMI had approximately 5 times lower 50% lethal concentration (LC50) than IMI in zebrafish embryos, while no significant difference was observed in invertebrates. Through toxicokinetic modeling, no difference in bioaccumulation was observed between nano-IMI and IMI in *D. magna* based on one-compartmental toxicokinetic model. A two-compartmental model successfully simulated the slow elimination of IMI from *C. kiinensis* and confirmed that both pesticides reached toxicologically relevant targets at saturated levels. In addition, due to the unintentional partitioning, zebrafish chorion was detached to elucidate organismal uptake. Nano-IMI exhibited significantly higher bioaccumulation potential than IMI in zebrafish embryos, explaining the higher toxicity observed. Furthermore, the freshwater ecotoxicity of pesticides from

production to the end-of-life emission were predicted synergistically using life cycle assessment, USEtox and SimpleBox4Nano models. The ecotoxicity characterization factor for IMI ranges from 1.25E+04 to 3.29E+05 CTUe under various scenarios considered, which is higher than that of nano-IMI (3.76E+03 CTU). Although with a relatively higher ecotoxicity in fish embryos, our study demonstrated that the substitution of IMI with nano-encapsulated IMI is anticipated to mitigate their life cycle freshwater risks. In conclusion, the present study provides a comprehensive understanding of nano-IMI's environmental risks and its potential impact on freshwater ecosystems compared to IMI. Nano-IMI may not always be safer than its conventional counterpart, but its use may be more sustainable if proper management measures are implemented during its production and application.

#### **6.08.P-Th550 Assessment of Innovative Polyurethane Coatings: Anti-Corrosion Performance and Ecotoxicological Effects**

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Corrosion mitigation poses significant economic and environmental challenges, mostly due to the use of protective coatings containing toxic corrosion inhibitors (CI), that are released to the seawater during the coating's life cycle. Thus, a new generation of nanostructured bio-based CI, less toxic than the widely used compounds, has been developed in the framework of the NANOGREEN project, using layered double hydroxides (LDH). LDH are a class of anionic-exchange nanoclays characterized by positively charged metal (e.g., Zn<sup>2+</sup>, Al<sup>3+</sup>) stabilized by anions (e.g., NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>). Such nanostructured forms (LDH-Gluconate, LDH-Glutamate, in slurry, or powder) and biobased CIs soluble (gluconate, glutamate), were used as anti-corrosion additives in a model polyurethane (PU) coating. This study aims to assess these novel PU coatings' anti-corrosion efficacy and safety. Anti-corrosion properties were analyzed over 21 days using electrochemical impedance spectroscopy. Coated plates (~43cm<sup>2</sup>) were then immersed in artificial seawater for 84 days, and leachate samples were collected periodically for chemical and ecotoxicological tests. Metals (Zn and Al) were analyzed using ICP, and key anions/CIs (nitrites, nitrates, chloride, gluconate, glutamate) were analyzed using HPLC and ionic chromatography. Ecotoxicological tests involved microalgae (*Tetraselmis chuii* and *Phaeodactylum tricornutum*), a rotifer (*Brachionus plicatilis*), and a crustacean (*Artemia salina*). Multivariate data analysis using Principal Coordinates Analysis evaluated the temporal trends in ecotoxicological data and their correlation with environmental descriptors (chemical and physicochemical data). Globally, the PU coatings exhibited good anti-corrosion properties, including satisfactory adhesion and barrier protection, better in the case of the nanoadditives-based coatings. Leachates from LDHs/PU coatings showed no significant toxicity on the tested species. Released metals and anions had a limited impact since pH and conductivity were the most correlated environmental factors with the ecotoxicological data. This suggests that the concentrations of metals, anions, and biobased IC were insufficient to cause ecotoxicological responses, indicating minimal release from the coatings over the 84 days. The new bio-based nanoadditives PU coatings showed good performance for maritime applications with no/low environmental impact, which can mitigate the early leaching of conventional corrosion inhibitors.

#### **6.08.P-Th551 The dampening impact of the dual transformation of ENPs for their phytotoxicity**

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The galloping production and usage of engineered nanoparticles (ENPs) entail their dissemination in the environment. Once ENPs are released into the environment, they undergo physical, chemical, and biological transformations, altering their properties. The environmental-induced modification of ENPs is dynamic and once transformed ENPs may be the subject to subsequent processes. In this regard, the properties of transformed ENPs (*trans*ENPs) will differ from the pristine ones (*p*ENPs), and finally, it may determine their fate and toxicity. The goal of this study was to assess the phytotoxicity of pristine heavy metal-based ENPs (ZnO, CuO), single (sulphidised) and double-transformed (sulphidised covering with protein corona). The properties of *p*- and *trans*-ENPs, including the transformation rate, were characterized in terms of the phase composition, size, zeta potential, crystallinity, size of surface area, and dissolution rate. The toxicity of ENPs was evaluated using the seed germination and root elongation test on *Lepidium sativum*. The more transformed ENPs revealed lower toxicity compared to sulphidised or *p*ENPs. The higher mitigation in phytotoxicity under transformation was noted for the CuO ENPs than ZnO ENPs. The attenuated toxicity could be related to the decreased concentration of Zn or Cu ions in the exposure to *trans*ENPs. The effects may also result from the distinct interactions (mostly tend to adhesion) between ENPs and the surface of seedlings. The obtained results first reported the decreased phytotoxicity of chemically-biologically transformed ENPs compared to pristine nanomaterials. Further studies on the chronic effects of modified ENPs are requested to gain the full picture of their fate in the environment.

#### **6.08.P-Th552 Ecotoxicity Tests of TEMPO Oxidized Cellulose Nanofibers**

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Cellulose nanofibers (CNFs) are novel nanomaterials derived from plants. Based on their inherent attributes, which include

high strength, high water retention characteristics, and transparency, CNFs have been extensively applied across diverse fields, such as pharmaceutical products, cosmetics, food thickeners, automotive engineering, reinforcing tire, household electrical appliances, and house construction materials. As such CNFs are expected to contribute to a sustainable society and ongoing technological advancements have been made to increase their practical applications. An increase in the mass production of CNFs could result in increased material disposal into the environment, leading to potential ecological effects on aquatic organisms. Hence, the effects of CNFs on aquatic organisms should be evaluated in parallel with the development of CNF. However, there is little information available on the aquatic ecotoxicity data of CNFs. nanomaterials such as CNFs are treated as difficult-to-test substances in existing guidelines. For example, the properties of CNFs, such as dispersibility and dispersion stability, are prone to alteration in response to changes in water quality. Without an understanding of the properties of CNFs in the test medium and the variation in the properties due to variations in test conditions, the ecotoxicity of CNFs cannot be adequately assessed. Thus, in this study, we have established the methods for ecotoxicity tests dedicated CNFs and get toxicity data for aquatic organisms. In this presentation, we will report the results of the ecotoxicity test method investigated and tested using algae, daphnia, and fish for 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) oxidized CNFs.

#### **6.08.P-Th553 Mesocosm-based Studies for Assessing Impacts of Nano-enabled Pesticides on Freshwater Ecosystems: Results & Opportunities**

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Recent agricultural and food policy reforms across the globe have placed a strong focus on the mitigation of environmental risks associated with pesticide use. Nano-enabled pesticides, i.e. pesticidal products containing nanoscale active substances and carrier systems, are increasingly proposed to possess properties which could aid in the attainment of such objectives. Experimental assessments of their potential favorability over non-nanoscale analogues to date has predominantly relied on their enhanced pesticidal- (i.e. target) efficacy and efficiency, and less so on their possible collateral environmental impacts. Such a focus is likely to skew evaluations with regards to their potential benefits towards the former. Furthermore, few studies up to now have evaluated non-target impacts of nano-formulated pesticides using mesocosm setups, which has resulted in a lack of understanding of their potential adverse environmental impacts at higher levels of ecological organization. We present an overview of findings from our recently published and currently ongoing studies concerning freshwater mesocosm-based assessments of nano-enabled pesticides, focusing on two different formulations. Of a (nano)TiO<sub>2</sub>-based formulation of carbendazim, we demonstrate impacts on a variety of taxonomy- and function based community parameters of macroinvertebrates and zooplankton, which could predominantly be attributed to effects induced by carbendazim. Of a (nano)clay-based formulation of clove essential oil, we demonstrate that abundances of emerged insects exposed during aquatic larval stages are partially attributable to the presence of the clay carrier, but that community parameters overall show minimal response to clove essential oil. Our findings illustrate the suitability and feasibility of mesocosm studies for the evaluation of risks and benefits associated with the use of nano-enabled pesticides. To facilitate future work in this regard, we use our findings to outline how we believe the selection of specific formulations and experimental designs can best be tuned to provide relevant and generalizable insights.

#### **6.08.P-Th554 Toxicity of Repeated Exposures to Electronic Cigarette and Heat-not-Burn Aerosols on Human Bronchial Epithelia: Combining Biological Assays and Microscopy Analysis**

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The use of electronic cigarettes (e-cigs) and Heat-Not-Burn (HnB) products is fast growing, raising concerns regarding their potential harmful pulmonary effects in former and current smokers. However, evidence-based data are still lacking for assessing the human health risk related to exposure to e-cig and HnB aerosols. Thus, the characterization of inhaled aerosols and the evaluation of their effects on the target tissue are necessary.

The present study aimed to evaluate the effects of repeated exposures of e-cig and HnB aerosols on normal human bronchial epithelia (HBE) using realistic in-vitro technologies. A complimentary goal was to improve our understanding of cytotoxicity effects using microscopic evaluation to strengthen our knowledge of potential adverse effects of e-cig and HnB aerosols on human airway cells.

Aerosols were produced from a Joyetech e-cig (third generation) and an IQOS HnB with a specifically developed aerosol generation chamber mimicking user puffing behavior. Air liquid interface cultures of reconstituted normal human bronchial epithelial cells (HBEC) were exposed to single or multiple puff topographies of e-cig aerosols, using the (nano)aerosol deposition chamber NACIVT. The Stepanizer software tool was used for semi-quantification of cell detachment on light microscopy images taken directly after exposure, at 4 h and 24 h post-exposure. Cell death (lactate dehydrogenase release LDH), and epithelial damage (electron microscopy) were evaluated at 4 h and/or 24 h after exposure to the aerosols.

Previous analysis showed that repeated exposures to e-cig and HnB aerosols caused cytotoxic effects in a dose-dependent manner. LDH levels increased with the number of produced puffs and were higher for Joyetech aerosol. Our semi-quantitative evaluation revealed epithelial damage by cell detachment of cells exposed to the first puff topography in a dependent manner

to deposited aerosol mass and post-exposure time. Ongoing analysis focuses on the evaluation of cell detachment following repeated exposures with an increasing number of puff topographies.

#### **6.08.P-Th555 Advanced Materials Earliest Assessment (AMEA)**

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Advanced materials are rapidly being developed in different material categories. They share little commonalities apart from their novelty, which raises concerns that these materials may fall into a regulatory gap with potentially inappropriate risk management. But how to assess materials that are still under development? Here we present the Advanced Material Earliest Assessment (AMEA) approach to fill this gap by proposing simple assessment steps and guidance for design rules being applicable by innovators in early material development phases (ideation, business case and lab phases). AMEA provides a structured approach to exploit the available knowledge at each phase, starting from the intended product, application and global region, starting also from the conventional material in the same application, of which the sustainability benefits and sustainability challenges often constitute the motivation for advanced material development. During the lab phase, AMEA recommends focusing on acquisition of data by selected New Approach Methods (NAMs) with discriminating power, and triggers *more requirements* and/or *specific testing methods* depending on the positioning of the material with respect to the three dimensions “nano-enabled?”, “advanced?”, “containing particles?” The methodological part can be amended for other material classes without relevance of nanostructures. Similarity and ranking approaches compare material versions synthesized in lab phases against each other and the conventional material in terms of performance, lifecycle emissions/exposures and hazards. AMEA prioritizes the discriminating power of specific data to refine the design targets instead of using generic assumptions with high uncertainties.

AMEA is currently being implemented as an online tool that provides safe-and-sustainable-by-design (SSbD) prioritisation and guidance for each of the three innovation phases based on the categorization that follows answering the three main questions (<https://diamonds.tno.nl/projects/harmlesspublic>). It is the entry point of the HARMLESS decision support system which covers also the ensuing pilot and launch phases of innovation management to implement SSbD material development.

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#### **6.08.P-Th556 Cellular Responses of Eisenia fetida Coelomocytes Exposed to Wastewater Treatment Plant-Transformed Isotopically Enriched Nanomaterials**

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Most nanomaterials (NMs) used in commercial applications enter wastewater streams and reach wastewater treatment plants (WWTP), where the vast majority is concentrated in sewage sludge and applied on agricultural land. By the time NMs enter soils, they have undergone various transformation processes, which results in particles with altered properties, bioavailability and toxicity which can differ from the original pristine NMs. While less documented than for pristine NMs, the environmental hazard of environmentally relevant chemical forms of NMs is critical knowledge for risk assessment. The aim of this study was therefore to assess the potential environmental hazard of WWTP-transformed NMs in environmentally relevant exposure conditions.

To be able to trace low concentrations of NMs in complex matrices like sewage sludge and soil, with high natural metallic background, isotopically enriched <sup>109</sup>Ag, <sup>46</sup>TiO<sub>2</sub> and <sup>68</sup>ZnO NM were synthesized and introduced to a pilot WWTP. The resulting sludge was applied to soil microcosms with *Eisenia fetida* earthworms, and the potential toxicity of the transformed NMs was assessed in coelomocytes isolated from the exposed earthworms. Coelomocytes are primary immune cells involved in phagocytosis, encapsulation and humoral immune responses, sensitive towards metal and NM pollution, and thus a promising method for high-throughput assessment of NM immune-related effects.

NM characterization was performed with dynamic light scattering (DLS), single particle-Inductively Coupled Plasma Mass Spectrometry (sp-ICP-MS) and transmission electron microscopy (TEM). The NM-containing sludge was characterised by ICP-MS using a developed isotopic ratio method. The effects of the sludge containing aged NM on the coelomocyte population, metabolic activity, lysosomal integrity, reactive oxygen species formation, mitochondrial membrane potential and immune responses were assessed and compared to control (non-spiked) sludge after 1 and 4 weeks of exposure. Nanoparticle uptake and intracellular localisation were evaluated with sp-ICP-MS. The importance of taking NM transformation into

account and the sensitivity of the *E. fetida* coelomocytes as a cell model to study immune-related responses of transformed NMs are discussed.

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#### **6.08.P-Th557 In Vitro to Organ Level Effect Extrapolation for Human Risk Assessment of Nanomaterials**

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Nanomaterials exhibit distinctive properties at the nanoscale and have become integral to diverse industrial sectors, including energy, food, and medicine. Despite their transformative impact, growing concerns surround the potential adverse effects of nanomaterials on human health and the environment. This study investigates the application of risk assessment to identify and quantify the risks associated with nanomaterial release, with a particular focus on the challenges posed by limited *in vivo* studies due to ethical considerations. Risk assessment serves as a tool to identify and measure the risks associated with the release of nanomaterials into the environment and the subsequent exposures to humans and ecosystems. However, in the field of human nanotoxicology, we have the challenge that only few *in vivo* studies are available. The emergence of new nanomaterials further complicates the timely calculation of dose-response assessments. *In vitro* analysis provide a promising alternative to obtain hazard data for nanomaterials, but their integration into risk assessment remains in its infancy.

This research investigates strategies for extrapolating toxicity data from *in vitro* studies to organ-level effects. The approach involves the development of a combined dosimetry model that integrates *in vitro* dosimetry with *in vivo* dosimetry (Physiologically-based pharmacokinetics model). This model aims to predict safety levels for human nanomaterial uptake, translating cellular-level internal doses into concentration levels that trigger organ reactions. The focus is particularly on the liver, which accumulates some of the highest concentrations of all organs. When faced with diverse *in vitro* nanotoxicity datasets, Bayesian statistical analysis emerges as a valuable tool for characterizing uncertainties arising from inter-experimental variations. This study not only contributes to advancing our understanding of nanomaterial toxicity but also provides insights into assessing the influence of experimental result variability on extrapolating safe nanomaterial concentration levels for humans. It effectively bridges the gap from *in vitro* studies to prediction of organ-level effects in the context of human risk assessment, contributing to the comprehensive evaluation of nanomaterial hazards.

#### **6.08.P-Th558 Safety Assessment of Hexagonal Boron Nitride at the Skin Level: an In Vitro Study on 3D Reconstructed Human Epidermis and HaCaT Keratinocytes**

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Hexagonal boron nitride (hBN) is a novel two-dimensional nanomaterial, constituted of boron and nitrogen atoms, that is currently developed in many technological applications, ranging from electronic to biomedicine. However, the enhanced use of hBN needs a careful evaluation of its impact on human health. Skin contact can be considered one of the most relevant exposure routes to this material not only in occupational settings but also considering a final user exposure.

To investigate the cutaneous effects of hBN, NAMs for skin irritation and corrosive properties assessment were applied in compliance with the Organization for Economic Co-operation and Development (OECD) test guidelines 439 and 431, respectively, using an *in vitro* advanced 3D model of reconstructed human epidermis. When applying these test guidelines, hBN resulted a non-irritant and non-corrosive material at the skin level. Moreover, it possessed also an extremely low pro-inflammatory potential.

Subsequently, an *in vitro* toxicity study of hBN was carried out on HaCaT cells, an immortalized human keratinocytes cell line. hBN was able to be massively internalized by keratinocytes; however, it induced a significant reduction in cell viability and mass exclusively at very high concentrations (>50-100 µg/mL) only after 72 h exposure. In the effort to explore the mechanisms underlying its cutaneous toxicity, even if low, hBN was assessed for its ability to alter a series of cellular parameters. In particular, the disruption of plasma membrane integrity induced by hBN appeared associated to a mitochondrial damage causing a significant reactive oxygen species production.

In conclusion, these results demonstrate the lack of irritation and corrosive potential of hBN but also its ability to induce very low cytotoxic effects on HaCaT keratinocytes, suggesting its good biocompatibility at the skin level.

#### **6.08.P-Th559 Global RNA Sequencing of Escherichia coli from Exposure to Nanodiamonds and Carbon Nanotubes with Surface Coatings**

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Engineered nanomaterials can be tailored with specific physico-chemical properties to satisfy required commercial, medical or any other need. These materials can drive innovation as a result of the infinite chemical structures that can be produced. There are also concerns that these materials will negatively affect microbes that inhabit end-of-pipe ecosystems. Global RNA sequencing was used to describe gene expression levels, as messenger RNA abundance, in response to different test exposures. Global RNA sequencing is valuably used to describe gene expression levels, as messenger RNA abundance, in response to different test exposures. This has been made easier through fast genomic sequencing and the identification of differentially regulated genes in response to stimuli. Such approaches, despite still being immensely costly, can be used to refine animal testing and provide a basis for non-animal methods of hazard assessment with nanomaterials. The bacterium *Escherichia coli* K-12 MG1655 was used as this organism is well annotated in molecular databases and has environmental significance. The materials included in the study were nanodiamonds and multi-walled carbon nanotubes with carboxylate, amine and polyethylene glycol coatings, respectively. This research aimed to see if changes in RNA expression reflect the toxicity ranking of these materials from *in vivo* results. Sub-lethal exposure concentrations were necessary such that adaptive molecular responses could be identified, rather than the omics of a dying organism. All test suspensions were characterised for particle number concentration and particle size distribution. The increases in RNA expression relative to the unexposed control generally reflected the toxicity ranking from the *in vivo* results. The biggest RNA increases were related to genes involved in monosaccharide transport (*Isr* genes), glycolate metabolism (*glcE*) and lactate transporters (*glcA*). Global RNA sequencing detected differences in gene expression that suggest material-type effects and also specific coating effects within these nanomaterials. ENMs. However, these observations were not so evident *in vivo* with growth as an endpoint.

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#### **6.08.P-Th560 Beyond Conventional Measures: Embryotoxicity Evaluation of Innovative Nanostructured Corrosion Inhibitors**

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Metallic corrosion has been mitigated through specialised coatings containing efficient but often toxic corrosion inhibitors (CIs), such as 2-mercaptobenzothiazole (MBT). Recent developments have proposed using engineered nanomaterials, like layered double hydroxides (LDH), to immobilise CIs, such as MBT (abbreviated as LDH-MBT), for controlled release with environmental benefits. However, the long-term effects are scarcely understood. In this context, the current study focused on the embryotoxic effects of LDH-MBT, as well as unloaded LDH (Mg-Al LDH-NO<sub>3</sub>) and MBT in the soluble form, using the sea urchin *Paracentrotus lividus* as a model organism. Unloaded LDH was categorised as not toxic (EC<sub>50</sub>>100 mg/L). In contrast, both forms of MBT were categorised as very toxic, with EC<sub>50</sub> values estimated at 0.49 and 0.93 mg MBT/L for MBT and LDH-MBT, respectively, and presented a similar NOEC value (0.137 mg MBT/L). However, detailed observations of embryo-larval development revealed distinct effects between the soluble and nanostructured MBT forms. For instance, embryo development was interrupted at the first cell divisions at 33.3 and 100 mg MBT/L of soluble MBT, whereas the morula stage was reached with the corresponding concentrations of LDH-MBT. Furthermore, at 11.1 mg MBT/L, the morula stage was observed for MBT, whereas the blastula stage was reached for its nanoform. At 3.7 mg MBT/L, embryos reached gastrula and prism stages in LDH-MBT treatment, compared to the morula and blastula stages observed at the same concentration for soluble MBT. At 1.23 mg MBT/L, animals reached the pluteus stage for both forms of MBT. Therefore, this study emphasizes the need for a more comprehensive strategy using the embryo-larval responses. The analysis reveals that, although the LDH-MBT is classified as very toxic, the methodology employed in this study evidenced a retard effect, probably related to the controlled release capacity of this innovative nanoform which limits the exposure concentration of soluble MBT over time. These findings highlight that a mere classification of toxicity based on traditional analysis is insufficient. It underestimates the comparative severity of impacts, indicating a need for a deeper understanding of the embryo-larval developmental effects as a sensitive endpoint to ensure an environmentally responsible application of these innovative materials.

#### **6.08.P-Th561 Differentially induced autophagy by engineered nanomaterial treatment has an impact at cellular homeostasis and cytotoxicity**

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Considering the escalating production of engineered nanomaterials (ENMs), hazard needs to be carefully evaluated in order to foresee detrimental future consequences for human health and the environment. In order to reduce animal testing, New Approach Methodologies (NAMs) emerge as promising tools, but the development of effective NAMs necessitates a thorough understanding of ENM's mode of action (MoA). In this work, a detailed set of *in vitro* tests has been constructed to facilitate the categorization of 10 ENMs by unraveling their mode-of-action with the help of *in chemico* assays. Cell viability testing via WST-1, LDH release estimation of cell permeability, autophagosome detection, and proteomics are employed to study toxicity in lung epithelial A549 and differentiated THP-1 (dTHP-1) macrophage-like cells, which has been correlated with the reactivity of the nanomaterials' surface. For instance, non-reactive nanomaterials like SiO<sub>2</sub> NM-200 do not affect cell viability. In contrast, highly reactive ENMs like CuO and ZnO (NM-110 and NM-111) substantially impact the viability of A549 and dTHP-1, leading to a loss of homeostasis. Yet, unique protective mechanisms emerge for moderately reactive ENMs: we have observed for the first time that TiO<sub>2</sub> (NM-101 and NM-105), known for its acidic behavior, and CeO<sub>2</sub> (NM-211 and NM-212),

recognized for its oxidative surface reactivity, prompt the formation of autophagosomes. This is evidence of autophagy induction, which appears to be activated as a protective measure against the MoA of these reactive ENMs, helping to maintain cell homeostasis.

#### **6.08.P-Th562 Safer by design approach to support innovation: a practical case study with polymers**

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Solvay aims to develop different activities to assess the potential impact of substances during their manufacturing, their use or their degradation/release. A safer-by-design approach enables the selection of the best candidates from application performance tests, (eco-)toxicological assays and biodegradability results. These approaches are employed during the development of a new range of safe and sustainable products by our Research and innovation function.

As part of the safer by design approaches, we carry out toxicological evaluations at an early stage of development. In the context of the minimization of animal experimentation with the 3R strategy (Reduce, Reuse and Replace), the development of *in vitro* evaluation protocols is attractive, at least for the initial phases of toxicological assessments for new substances. Therefore, Solvay uses and develops *in vitro* models relevant to assess typical exposure routes (*i.e.* pulmonary, cutaneous, intestinal systems) associated to expected final end-markets of market end points.

Among the materials which have gone through this process during the past years, some polymer case studies are presented here. Polymers are widely used in various materials, consumer or industrial applications such as paints, household and personal care formulations, packaging etc. with increasing human exposure and thus the potential risks related to short- and long-term toxicity for both consumers and workers. Today, polymers are not REACH registered, but the reacted monomer and the other substances that form part of it, must be registered if their concentration exceeds 2% (w/w) or if their quantity exceeds 1 tonne per year. Considering these uses and the diversity of polymers, innovative methods able to predict and assess effects at environmental release and exposure level are required.

The battery of tox evaluations presented here include a catalog of cell lines mimicking different organs and an exposure up to 100h using real time monitoring of cell proliferation allowing a direct comparison between the candidates but also to compare observed effects toward a control (non exposed condition).

#### **6.08.P-Th563 Biological Effects of Microcapsules**

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Microcapsules are the minute particulate matter which encloses various chemical substances inside of the core. The outer shells are often made by a kind of plastics so it could be regarded as microplastic.

They are used in many aspects including drug delivery, stabilizing the agricultural chemical effects and to keep fragrance durability of the softening agent.

The behavior of the chemical in the capsules changes depending on the properties of outer shell.

It means the characteristic of microcapsules such effect like easily decomposable chemicals to stable or volatile component to less diffusible, provides the change to the behavior of encapsulated chemicals, may cause the hazard in the environment.

Encapsulation allows any chemical substance to have long-distance mobility and resistance to decomposition, and may have properties equivalent to so-called POPs. Therefore, with the aim of clarifying the ecological effects of chemicals mediated by microcapsules, we synthesized microcapsules ourselves and investigated changes in the toxicity of chemicals before and after embedding them in capsules, and here we report.

As for the toxicity test, a *Daphnia* acute toxicity test in accordance with OECD Test Guideline 202 or a fish acute toxicity test in accordance with OECD Test Guideline 203 was conducted. Capsule shape and number were determined using an optical microscope and a particle counter.

#### **6.08.P-Th564 Application of Adverse Outcome Pathway framework in assessing nanogold exposure to *Daphnia magna***

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Nanotechnology has permeated almost every aspect of technology, science, and daily life, and it is a promising field for future advancements. Nanomaterials (NMs) are materials with a one-dimensional size range of 1-100 nm and can exist in a variety of elemental cores and shapes with varying surface coatings. The developmental of nanogold (nAu) has become advantageous as it is applicable for medicinal diagnosis, imaging and as a drug delivery vector. When NMs are released into the aquatic environment, they have the potential to harm aquatic organisms at all stages of their product lifecycle. However, there is little known about the harmful effects of these NMs, particularly at the molecular level. The adverse outcome pathway (AOP) framework was utilized in this study to address adverse effects of nAu (CTAB capped rod shaped nAu and citrate capped spherical nAu) and ionic Au on *D. magna* at different levels of biological organisation using M7 media. The molecular

initiation event was determined at the molecular level using metabolomics, Key Events at the whole organism level using physiological changes based on swimming behaviour, heart rate, and respiration and an Adverse Outcome at community level (reproduction and functional response). CTAB capped rod shaped nAu with a size of  $\pm 40$  nm had an LC50 of 12.1  $\mu\text{g/L}$ , the highest acute toxicity of all groups tested. Ionic Au had an LC50 of 57  $\mu\text{g/L}$  while spherical citrate capped nAu had a size of  $\pm 20$  nm had an LC50 of 70  $\text{mg/L}$ . Several metabolic pathways were affected after 48 h exposure and these included the biotin metabolism, insect hormone biosynthesis, amino acid metabolism and nicotinate and nicotinamide metabolism. CytoViva darkfield imaging confirmed adhesion of nAu to the carapace of daphnids and higher concentrations were observed on molts. The heart rate increased significantly at the LC20 for all groups, while respiration increased for nano-exposed groups and decreased for the ionic Au group while reproduction was significantly decreased compared to control for all groups. The behaviour of daphnids was significantly altered between groups where citrate capped nAu had increased molting and remained mobile in the bottom zone, while CTAB and ionic Au were found significantly more within the top zone. The functional response tests indicated a decreased escape response in daphnids, and therefore survival. Nano-specific effects were observed in two types of nAu when compared to their bulk ionic counterpart.

#### **6.08.P-Th565 Two-dimensional Hexagonal Boron Nitride (h-BN) and One-dimensional BN Nanotubes (ID-BNNTs): Structural Analogs to Graphene and Carbon Nanotubes with Distinct Effects**

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Through tailoring of nanomaterials the future will see more advanced and 2D materials emerging. For example, h-BN's honeycomb hexagonal atomic structure is isomorphic to graphene, while BN nanotubes (BNNT) are analogous in shape to carbon nanotubes. Besides the similar form there are unique properties conferred upon these materials, by replacing carbon with boron and nitride atoms, that make them very attractive in a wide range of human activities. Expanding our knowledge about the particularities of the interaction of these materials with cellular systems is essential for an appropriate risk assessment of these substances, but also for the application of safe and sustainable by design (SSbD) frameworks and for the use of grouping and read-across approaches.

In order to generate data about possible environmental effects of h-BN and BNNT, the rainbow trout (*Oncorhynchus mykiss*) was used as a model aquatic test organism and a range of cell lines from the same species: *RTgill W1*, *RTG-2*, *RTH 149*, *RTL W1*. Effects on cellular metabolic activity, plasma membrane integrity and lysosomal functioning were characterised and any increases in reactive oxygen species (ROS) were compared and contrasted. While h-BNs were visualised enclosed in endosomes, BNNTs were observed free in the cells cytoplasm with no apparent mechanism of uptake or intracellular processing. BNNTs caused a dose dependent reduction in cellular metabolic activity in all cell lines, and to a similar extent, while no effects were evidenced for h-BN. Instead h-BNs only caused effects on the plasma membrane, and while increases in ROS levels were measured this did not lead to a reduction in cellular metabolic activity. Effects on lysosomal functioning could not be interpreted due to interference of the materials with the neutral red dye used in this assay.

These findings highlight that apart from elemental composition, material form and atomic arrangement play a decisive role and will dictate the extent of advanced materials interactions with biological systems. As material science expands into the 2D materials space with endless possibilities of atomic replacements and combinations, cellular *in vitro* testing platforms can provide key mechanistic focused information in SSbD frameworks.

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#### **6.08.P-Th566 Comparing the Effects of 6PPD and a Mixture of Atmospheric Transformation Products on Immortalized Chicken and Double-Crested Cormorant Hepatic Cell Lines**

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*N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine (6PPD) is an antioxidant added to tires to prevent degradation. 6PPD reacts with atmospheric oxidants and creates transformation products either on tire surfaces or on particles emitted into the atmosphere. One of its transformation products, 6PPD-quinone, has been found to be toxic to aquatic organisms, especially coho salmon in the Pacific northwest. However, little is known regarding the toxicological properties of 6PPD and/or its transformation products (TPs) and mixtures of TPs in birds. In this study, cell viability, reactive oxygen species (ROS), oxygen consumption rate, and changes in mRNA expression will be determined in immortalized chicken and double-crested cormorant hepatic cell lines, LMH and DCH22, cultured as 3D spheroids following exposure to 6PPD and four mixtures of TPs formed under various typical atmospheric conditions. Screening 6PPD and the mixture of TPs will provide improved understanding on the effects of these compounds given the large differences in sensitivity between aquatic species. The TPs were generated in the presence of ultraviolet radiation (UV), ozone (O<sub>3</sub>), hydroxy (OH) radical, and nitrogen oxides (NO<sub>x</sub>; in the presence of OH) using a custom oxidation flow reactor. Neat extracts were dissolved in DMSO, and serial dilutions were prepared in DMSO. Cell viability and ROS were evaluated using intracellular ATP concentration and oxidation of 2,7-dichlorofluorescein diacetate (DCFDA) fluorogenic probe by ROS, respectively. Customized species-specific PCR arrays comprising 48 genes

will be used to determine changes in mRNA expression. The O<sub>3</sub> and UV TPs were the most cytotoxic and caused an increase in ROS in LMH spheroids. The OH and NO<sub>x</sub> TPs had similar cytotoxicity as 6PPD (LC<sub>50</sub> values between 0.09-0.18) in LMH spheroids and all three mixtures increased ROS concentration. The decrease in ATP concentration and increase in ROS by 6PPD and the TPs suggest impairment of mitochondrial function in LMH spheroids. The rate of oxygen consumption in the spheroids will elucidate more information on mitochondrial toxicity, while gene expression data will provide information on the mechanism of toxicity for 6PPD and the TPs. Based on the prevalence of these compounds, this study will generate much needed toxicity data for avian species.

#### **6.08.P-Th567 Instantaneous photocatalytic degradation of pesticides over coupled ZnO@CdS nanocomposite via greener approach**

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Photoactive ZnO@CdS coupled nanocomposite was designed by *Azadirachta indica* extract via greener mode. Consequently, the targeted pesticides chlorpyrifos (CP) and atrazine (ATZ) have high poisonous and persisting behavior in the environment. Different spectroscopic and microscopic techniques were performed to characterize the coupled nanocomposite. Crystalline behavior and irregular shape with 50nm particles size was suggested by PXRD and FE-SEM analysis, respectively. New spectroscopic vibration of Zn-S and Cd-O rooted the coupling of parent nanomaterials. The photo-catalytic activity of ZnO@CdS for degradation of pesticides was monitored at different concentration of pesticides, catalyst dose, variable pH parameter and irradiation source (light and dark). The large extent of degradation (89-91%) to visible region was due to reduction in band gap (1.67 eV), enhanced surface area (111m<sup>2</sup>g<sup>-1</sup>) and diminished ionic species recombination capability. The generation of photo active radicals was confirmed by scavenger analysis. The Effect of ionic strength was helpful to understand the interaction mechanism involved in the removal of contaminants. Being more effective than natives, ZnO@CdS has substantially suppressed the half-life of pesticides as revealed from generation of smaller and less toxic metabolites. The generation of smaller and non-toxic byproducts was analyzed by GC-MS technique. Langmuir model and first-order kinetics followed by photocatalyst in degradation process. Robust and photocatalytic behavior of ZnO@CdS upto 10<sup>th</sup> cycle could deep-rooted its sustainability high efficiency for environmental and industrial purposes.

#### **6.08.P-Th568 Co(II) oxidation to Co(III) at magnetite surface under oxidizing conditions**

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Magnetite (Fe<sub>3</sub>O<sub>4</sub>) and maghemite (Fe<sub>2</sub>O<sub>3</sub>) nanoparticles are widely used in environmental applications, particularly for soil and water decontamination. However, the interactions between metal ions and magnetite are not yet well understood, especially for transition metals. Our recent work<sup>1</sup> allowed to identify the formation of different Co(II) species at the surface of stoichiometric magnetite (Fe(II)/Fe(III) = 0.5). However, magnetite nanoparticles are most often used under oxidizing conditions. Therefore, the influence of oxidation on Co adsorption on oxidized magnetite (with Fe(II)/Fe(III) ratio = 0.1) was here studied.

Magnetite nanoparticles were synthesized by co-precipitation of iron salts under anaerobic conditions, then oxidized with H<sub>2</sub>O<sub>2</sub>. Co adsorption kinetics were performed under aerobic and anaerobic conditions, and revealed different adsorption behavior, with instantaneous adsorption under aerobic conditions and longer under anaerobic conditions. Batch adsorption studies were carried out under oxidizing conditions, with Co concentrations ranging from 0.1 to 12 mM, at pH 8, at room temperature. Some of the samples were filtered to determine the extent of Co removal from the solution. The solid phase was analyzed at SOLEIL synchrotron by X-ray spectroscopy (XAS) and X-ray magnetic circular dichroism (XMCD) at Co L<sub>2,3</sub>-edge. Furthermore, Co-magnetite reaction kinetics were followed by Quick-XAS at Co K-edge and multivariate curve resolution with alternating least-squares fitting (MCR-ALS).

Batch adsorption kinetic of Co onto magnetite drastically differed between aerobic and anaerobic conditions. Three Co species could be differentiated over time after MCR-ALS treatment of the Quick-XAS data. The first one, characteristic of Co(II), rapidly vanish, while the third one corresponds to Co(III) and, therefore, to the oxidation of Co(II) to Co(III). These results are supported by those obtained on the DEIMOS line, where the spectra reveal, once the reaction is complete, the presence of a mixture of Co(II) and Co(III) on the surface of the magnetite, with a larger proportion of Co(III) at high [Co]. The second species, difficult to determine, might therefore be a mixture of oxidized Co(II) and precipitated Co(II).

This study highlights the formation of mixed species of Co(III) and Co(II) precipitated on the magnetite surface, depending on the initial Co concentration, which might play a role in Co behavior and fate in oxic environments.

#### **6.09 Plastic Pollution: Bridging the Gap Between Science and Policy Needs**

##### **6.09.T-01 Experimental Assessment of Plastic and Biota Removal by Plastic Clean-up Mechanisms**

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Due to mismanaged plastic and its accumulation in the environment, various technologies have been developed and deployed to collect legacy plastic. However, because of the novelty and multitude of these technological mechanisms (i.e., the mechanical processes by which litter is gathered to a collection point, to be collected), their positive and negative impacts on the environment are, so far, largely unknown. As plastic clean-up technologies play a significant role in minimizing plastic litter in the environment, and as the United Nations Environment Assembly is working towards a legally binding and international treaty to target plastic pollution by 2024, empirical data are needed to guide and regulate the use of these technologies to ensure their net benefits. Since numerous parameters determine the collection of plastic and biota by plastic clean-up technologies, this study aimed to experimentally investigate the effects of four parameters (i.e., flow velocity, biota shape, plastic type, and plastic load) on the recovery rates of plastic and biota. Without intending to mimic the exact design of any specific company, we tested two independent, generic, and non-commercial plastic clean-up mechanisms, which we custom-built specifically for the experimental setup. The experiments were performed in a laboratory flume in which various conditions of each of the selected parameters were controlled and individually tested. For instance, three flow velocities (i.e., 0.1, 0.2, and 0.3 m / s) were separately assessed, while the other parameters were kept constant. Our preliminary results suggest that changes in flow velocity might have different effects on the recovery rate of biota. In a real case scenario, the removal of plastic or biota by a plastic clean-up mechanism is a combination of multiple parameters interacting with each other. Nevertheless, empirical studies such as this are necessary to establish baseline effects of each single parameter, contributing to the parameterization of hydrodynamic and ecological models.

#### **6.09.T-02 Predicting Plastic Degradation and Fragmentation in the Environment**

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The degradation and fragmentation of plastic in the environment is an important process that can have a significant impact on fate, exposure, bioavailability and ultimately risk. Therefore, future policy needs should consider the ensuing heterogeneity in sizes, shapes and degradation states. To enable this, prospective risk assessments must include information on plastic degradation and fragmentation, requiring the ability to make predictions of these across a range of environmental compartments and polymer types. In recent years, our mechanistic understanding of degradation and fragmentation has been developing, but until now, that knowledge has not been embedded into predictive modelling approaches. In this presentation, we introduce the FRAGMENT-MNP model – the first model to embed a mechanistic conceptualisation of plastic degradation and fragmentation, thus enabling realistic predictions of fragmentation and the subsequent evolution of size distributions for a broad range of polymers, environmental compartments and their environmental stresses.

The model conceptualises fragmentation rates, dissolution rates and fragment size distributions (the split of fragmenting mass amongst smaller size classes) that are functions of the degradation state of the polymer, polymer type, particle size, and mechanical power input. To parameterise the model, a comprehensive database of degradation and fragmentation experiments has been compiled. This includes a broad range of polymers (LDPE, PP, HIPS, PU, PET, PLA, and PA) that have undergone photolysis, hydrolysis and enzymatic hydrolysis at different temperatures and humidities, followed by mechanical disruption. We fit a machine learning model to these data in order to scale fragmentation rates, degradation rates and fragment size distributions to those corresponding to degradation stresses and mechanical powers likely to be encountered in the environment.

Our model is open-source and pragmatic in its data requirements, making it an easily usable tool for industry, regulators and academia, as well as being easy to integrate into exposure models. This offers a strong bridge between scientific understanding and potential future policy needs that take plastic heterogeneity into account.

#### **6.09.T-03 Leveraging Physiology & Behavior to Better Understand Exposure, Uptake, & Elimination of Micro- and Nanoplastics (MNP) in Pelagic & Benthic Species within the Context of Quantitative Risk Assessment**

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A significant body of ecotoxicological data have been developed for micro- and nanoplastics (MNPs). However, there remain challenges in synthesizing and interpreting these data within the context of ecological risk assessment. While many of these challenges are being addressed, particularly those around study design, QA/QC, and reliability, one significant challenge remains - identification of relevant species for use in the development of ecological protection criteria (i.e., PNECs, HC5). Previous studies have attempted to derive these values, however significant differences in species sensitivity have been observed for micro- vs. nano-sized particles. This results in (1) significantly different PNEC values as a function of the selected particle size / size range, and (2) uncertainty as to the relative sensitivities of different aquatic species to different MNP size, shape, and polymer types.

This work summarizes results of a systematic quality review of over 500 MNP biomonitoring studies using criteria previously developed by Hermsen et al., with minor modification to integrate relevance and reliability more clearly for risk assessment & prioritization of potential future environmental monitoring programs. For studies that met the criteria, trait-based descriptors and particle characteristics and distributions were compiled for all species to provide guidance on (a) species selection and (b) environmentally relevant exposure profiles for informing future biomonitoring and risk assessment studies.

The long-term objectives of this work are three-fold – first, to systematically compile and evaluate physiology and behavioral data for a wide range of freshwater and marine species into a searchable database for use in identification of sentinel species for ecosystem health & quality monitoring, and ecological risk assessment. Second, this work is intended to provide a biologically-relevant framework against which the relevance of existing (and developing) MNP reference materials may be evaluated and additional studies prioritized. This can provide a systematic basis for the inclusion or exclusion of species or materials for the purpose of quantitative risk assessment in various environmental compartments. Third, available information on ingestion and egestion rates as well as behavior and habitat can inform the selection of relevant and efficient species for integrated biomonitoring programs in sea surface, subsurface, estuarine, and sediment environments.

#### **6.09.T-04 Considerations Regarding the Representativeness of Microplastic Concentrations in Aqueous Systems: Are the Data Fit-for-Purpose?**

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A significant source of uncertainty in environmental exposure and risk assessment for micro- and nanoplastics (MNPs) stems from a lack of standardized methodologies and guidance related to sample collection strategies, extrapolation, and reporting of relevant computed values and their associated statistical variability. This lack of standardization further obfuscates the relevance and reliability of these data for use in the calibration and validation of quantitative exposure assessment models. The purpose of this work is to understand common sampling strategies, identify key parameters which may propagate uncertainty & variability in these techniques, and ultimately to develop a framework for systematically assessing relevance and reliability of these data for use in quantitative risk assessment of MNPs.

The development of guidance and best practices presented in this framework focus on several key areas for evaluation and categorization within a standardized rubric / system: (1) Synthesis and evaluation of existing methods for collection, quantifying, extrapolating, and reporting of environmental micro- and nanoplastic (MNP) samples, (2) communication of limitations & uncertainties of sampling strategies with respect to relevance and reliability (e.g., collection of samples by trawl vs. grab samples, sample volumes / trawl lengths, etc.), and their representativeness (e.g., limited geospatial or temporal scales) for informing an exposure assessment for the purposes of assessing risk, and (3) further identification and characterization of ancillary data collection and reporting metrics which are required / desired to increase the reliability and utility of existing and planned environmental MNP data (e.g., ocean current / river flow measurements, water chemistry, sampling depth, particle size range of sampler device, etc.). The relevance of data must be considered in the context of problem formulation and the purpose to which the data is put. We provide an approach to evaluate relevance of data and estimate representative sample volumes for a given study design, important aspects which can help with harmonisation efforts in this area.

#### **6.09.T-05 A little less conversation: How existing governance can strengthen the future global plastics treaty**

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The growing plastic production, the lack of their waste management, and fragmented regulatory responses have increased their abundance in the environment. Plastic pollution has created significant environmental concerns leading to planetary boundary threats. As a result, an increasing number of governments and non-state actors have begun negotiations on a legally binding treaty to cover the full-life-cycle of plastics by 2024. While the negotiations were mandated at the United Nations Environment Assembly 5.2 in March of 2022, how the new agreement would link to existing governance bodies addressing plastic pollution at the global, regional, national and local levels requires careful consideration. This analysis examines the main multi-level governance structures in place to govern plastics while highlighting their principal roles as well as shortcomings and gaps. It then explores ways a new global agreement could complement existing governance structures without imposing and duplicating the work of previous agreements.

#### **6.09.P Plastic Pollution: Bridging the Gap Between Science and Policy Needs**

##### **6.09.P-Mo508 Harmonizing beach litter data: A cornerstone for impact assessment**

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Plastic litter poses a global environmental problem, impacting marine and coastal ecosystems, human health, and societal well-being on a global scale. Plastic debris on beaches are an issue for wildlife, but also impacting cultural ecosystem services. Life Cycle Assessment (LCA) is one of the most used approaches for environmental assessments, but models to account for plastic impacts are scarce and for impacts of beach litter on cultural ecosystem services non-existent. However, the development of such a model is crucial for shaping policies and sustainable plastic management strategies.

Knowing the quantity of floating marine plastic that ends up on beaches is a prerequisite for any impact assessment model for beach litter. However, the unavailability or inconsistency of beach plastic litter reporting between countries makes this no easy task. Moreover, databases predominantly report the number of plastic items, rather than mass, which again may introduce variations in the data.

To address this issue, we investigated how big the discrepancies and potentials for harmonization between different reporting standards is and developed an approach to convert number of plastic items to mass. We collected data from 20 coastal countries and 8 years in Europe. Based on the composition of plastics item, the weight average of mass is calculated, either country specific or as a global average

With this knowledge impact assessment models for beach litter impacts on cultural ecosystem services can be developed, based on the willingness to pay for clean-up activities and this may help to develop appropriate policies for individual countries to combat coastal plastic pollution.

#### **6.09.P-Mo509 Towards a Safe Circular Plastic Economy: Using a Dynamic Probabilistic Material Flow Analysis Approach to Capture Japanese Plastic Flows**

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With the increasing production and consumption of plastics, a huge amount of waste is generated and subsequently discharged into the environment, posing unprecedented environmental challenges. The transition to a circular plastic economy is regarded as a solution to plastic pollution and resource depletion. Different from the traditional linear product lifecycle, a circular economy creates cycles where products, materials and chemicals are maintained in the cycle via reuse, recycling and so on. As one of the leading countries in recycling practices, Japan announced the Resource Circulation Strategy for Plastic in 2021, setting targets regarding the reduce, reuse and recycling of plastics. To achieve these targets, it is important to re-design the product lifecycle and develop a domestic circulation system to increase material efficiency. To do so, it is critical to first understand the current plastic use and waste management practices. However, the resolution of existing studies is not sufficient to provide such in-depth insights into the waste management of specific product categories, and a comprehensive investigation of all relevant industrial sectors and applications is missing. In this study, we model the domestic plastic flows in Japan since 1950, using a Dynamic Probabilistic Material Flow Analysis (DPMFA) approach and with a high resolution of the waste management systems. Plastic stocks in use and waste streams are estimated according to historical activity data and corresponding product lifetimes. The uncertainties of all parameters are evaluated using a data quality indicator matrix, and the model was solved 10,000 times following a Monte-Carlo method to present the variance of the model results. The model results present hotspots and blind spots of plastic losses from the technosphere, thus providing guidance on future research and action to enhance plastic circularity in Japan and other parts of the world.

#### **6.09.P-Mo510 Remobilization and Deposition of Plastics along Riverbanks in a Typical French Estuary During Complete Tidal Cycles**

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Estuaries are dynamic ecosystems that act as essential transition zones between freshwater and marine environments. They are considered to be areas of accumulation or reservoirs for plastics. The Slack estuary, a small estuary that flows into the English Channel at Ambleteuse (France), provides a remarkable example. Some plastics were collected in this estuary in order to define a set of 480 plastics of various types (shapes and polymers) and sizes, including microbeads (3-4 µm) and meso- and macroplastics (1 cm<sup>2</sup> to 25 cm<sup>2</sup>). Further, these plastics were then strategically placed along the banks in three types of quadrats with distinct substrates: gravels, sand and vegetation. The remobilization of these plastics was analyzed during six different campaigns, corresponding to dry and wet seasons, with high tidal coefficients and different environmental parameters (e.g. wind, current speed). Observations were made during two consecutive tides, leading to the monitoring of a complete tidal cycle. During this cycle, the plastics deposited by the tides were also observed. Our results showed that more than 90% of the manually deposited plastics were remobilized in water and that macroplastics were the least remobilized and that most of them remained in their substrates. For plastics that have not been remobilized, gravels and vegetation retained all types of plastic, with vegetation exhibiting the highest retention. Conversely, sand does not retain microbeads. This underlines the importance of substrate type in the remobilization of plastics. Moreover, negative correlations between environmental parameters and plastic remobilization were highlighted, underlining the complex interaction between ecosystem conditions and the distribution of plastics. After one complete tidal cycle, a deposition of  $2.6 \pm 2.07$  plastics/m<sup>2</sup> was observed. Macroplastics, especially fibers and ropes from maritime activities, seem to be key contributors to plastic pollution at the studied site. Finally, all the results showed the importance of understanding the remobilization and deposition of plastics along riverbanks in order to develop effective mitigation and remediation strategies.

### 6.09.P-Mo511 Exploring How AI Can Fill Data Gaps for Better Decision Making on Plastics Governance

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The omnipresence of plastics has led to policy makers asking questions about the environmental and health impacts of plastic use. These questions can be difficult to address as the specific composition of plastic products, packaging, and products containing plastics by different polymers is highly varied. Further, the volumes of plastics and their composition by polymers can differ across countries due to variations in economic sectors across countries. There is therefore a strong need for country-specific studies tracing the volumes of plastics from production through to end-of-life by economic sector for informed decision making. Mapping the flow and stocks of plastics through an economy can be challenging, however, due to data gaps. Possible data gaps include the composition of products by plastics, the composition of plastics by polymers, and on the lifetime of products and plastic components. In addition, trade data is not fit-for-purpose for country-specific mapping of plastic flows; the standardization of product groups across countries requires manual work to tailor the creation of groups that accurately reflect products traded in a specific economy. Here, we quantified the material flows and stocks of plastic polymers in the Norwegian economic sector using a static probabilistic material flow analysis (MFA). The data used in the model are a combination of trade statistics and data generated by Open AI's generative AI model Chat GPT-3.5 turbo (using a few-shot approach with fine tuning) where data gaps occurred. Specifically, AI generated estimates were used to estimate the compositions of product groups containing plastics by different polymers, the composition of packaging by polymers, and product group lifetimes. To test the robustness of these estimates, these were compared to data from random small samples of waste and national waste statistics. In this study, we found estimates provided from generative AI were broadly accurate and allowed for a more complete model of how plastics flow through the Norwegian economy. This study demonstrates that emergent AI technologies can allow us to fill data gaps to a certain extent for the creation of models tailored to specific contexts for more informed decision making.

### 6.09.P-Mo512 Paving the Way with a Holist Approach Combining Technologies and Actions to Reduce Litter in European Rivers

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The transport of litter, including macro-, meso- and microplastics, from rivers to the coast and ocean is of particular concern due to the potential ecological impacts of plastic litter in both freshwater and marine environments. The Innovative Solutions for Plastic Free European Rivers (INSPIRE) Horizon Europe project main goal is to significantly contribute to the reduction of litter through a holistic approach, that will enable the detection, collection and prevention of litter in river systems. To achieve this, our project will empower research and innovation, together with stakeholder and citizen participation, and blue investments. Through INSPIRE, we have enlisted collaborative efforts and know-how in disrupting plastic pollution, bringing together 20 technologies and actions in a consortium of 26 partners across Europe and Thailand. The project solutions include the implementation of strategies to i) collect litter from rivers (water, sediments, and riverbanks) using different technologies but also through organised cleanups with local citizens, ii) prevent litter by collecting and eliminating it from its waste streams before it reaches rivers and estuaries, and iii) develop alternatives for currently non-degradable polluting products (e.g., films used in agriculture and plastic packaging). Their technical feasibility and optimisation will be supported by cost-benefit and sustainability analyses and will include the development of business cases, strategic blueprints for scaling up and replication, and comprehensive mapping and modelling. A strategy will be in place for community engagement and dynamic communication, leveraged on multiple channels and tools to increase awareness locally and internationally. These elements converge to create a master plan to address the litter pollution related challenges at the European level and to contribute to the objectives of Mission 'Restore our Ocean and Waters' (European Commission). In this work, we share the INSPIRE innovative and collaborative approach by showcasing the six European rivers study cases where we are developing, deploying/installing, and testing innovative solutions. The complementarity of the technologic and behaviour-based solutions is explored, as well as how this concerted framework seeks to elucidate on litter pathways from rivers to the sea, paving the way for informed and evidence-based policy-making and mitigation strategies with the ambition to obtain plastic free European rivers.

### 6.09.P-Mo513 Mapping inland plastic flows in Santa Cruz, Galapagos

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Plastic demand is growing rapidly. Mapping of sources of plastic pollution is needed to inform intervention strategies. A major gap in such data exists for remote yet ecologically important locations such as the Galapagos archipelago. This study focuses



on mapping and understanding the anthropogenic factors influencing litter quantity in the most populated island in the Galapagos, Santa Cruz. First, we used data from products imported in 2022 to quantify the plastic input in the Galapagos Islands and the potential to become litter in a year. Then litter was collected in kerbside transects randomly selected to identify leakage of plastics inland. Furthermore, real time data of human movement was gathered using passive infrared sensors. To evaluate the influence of human movement (sensors data) with the quantity of litter found in each transect, we obtained a Spearman Correlation coefficient. Then the influence of the area type (urban, rural or protected) in litter quantity was studied using Kruskal-Wallis test, along with Conover-Iman test.

Results showed that food packaging contributes the most to litter generation in a year. This is not different from the data obtained in kerbside transects, where the most common items found were food plastic packaging. Moreover, the statistical analyses showed human movement had a weak influence on the quantity of litter found in kerbside transects. The quantity of litter items is significantly influenced by whether an area is urban, rural or protected. Further analysis will be performed regarding the different variables that can affect littering like the presence of industries, houses, parks, dumpsites, among others in the surroundings. These results aim to contribute towards informing interventions to reduce plastic waste, specifically in islands.

#### **6.09.P-Mo514 Environmental Fate of Plastic Pellets**

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Plastic pollution is increasingly threatening ecosystems' health. In the environment plastics are subjected to a range of degradation processes that lead to changes in their physicochemical properties and eventual breakdown into smaller pieces. The fate and ecological impact of aged plastics may well differ from that of their pristine counterparts.

We present a case study of the Scheldt estuary, located in northern Belgium, where the Port of Antwerp-Bruges, a large polymer hub for production, handling and distribution of industrial plastic pellets is located. Beginning decades ago, plastic pellets are being unintentionally released into the environment and finding their way to the Scheldt river. Measures are taken to prevent pellet loss but the problem is not yet solved. The environmental fate of the pellets is poorly documented and understood.

To identify the current extent of plastic pellet loss, 57 critical points on the port roads are being monitored using manual sampling (50'50 cm quadrant). Each season 5 samples are taken at each point over 2 weeks. Constructive communication with the companies and the port authority was the key to the establishment of this monitoring campaign. Analysis of the results reveals that pellet loss is ongoing in the port of Antwerp at an average rate of 3 to 4.5 plastic pellets per day per m<sup>2</sup> at the critical loss points.

To elucidate the environmental fate of the released plastic pellets, an extensive monitoring was set up. Pellets were manually sampled (50'50 cm quadrant) on the port road(side), on the Scheldt riverbank downstream and upstream. The spatial distribution of the number of pellets on the riverbanks reveals that more pellets are found upstream from the port than downstream. Physical and chemical properties, such as polymer type, chemical bond structure, morphology and surface roughness are being determined by Fourier-transform infrared spectroscopy, X-ray tomography, stereomicroscopy and scanning electron microscopy. The images reveal changes in colour and breakdown of the surface of pellets found on the riverbank.

Insights into the extent of pellet loss and the fate of the pellets provide an estimate of the current and future dynamics of the pellets in the harbour and estuary including the potential ecological risk. Results are shared regularly with the companies, which provides the opportunity to interact with managers and employees to implement best practises approaches towards zero plastic pellet loss.

#### **6.09.P-Mo515 Floating Barriers as a Monitoring Tool for Riverine Solid Litter: A Case Study in South America**

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This work aims to provide a tool for environmental monitoring of riverine solid litter contamination and potential input into the ocean, by presenting a new clean-up technology currently being used in the Portoviejo River in Ecuador. Large amounts of solid waste are discharged via rivers to world oceans due to a combination of increased production of anthropogenic materials and waste mismanagement. Observational data on the contribution of South American rivers to litter export to oceans currently lack on recent global estimates, which highlights the need for appropriate environmental analysis feeding on management and policy measures to reduce waste. To address this issue, Ichthion Limited (<https://ichthion.com/>) developed an extraction tool for riverine floating litter called the Azure system which is currently operative in the Portoviejo River (Ecuador) at 1° 01' 23.9" S, 80° 29' 35.6" W. Litter collected over two years of operation (2021, 2022) was weighed and classified according to their

probable source. Meteorological data retrieved from Ecuadorian National Institute of Meteorology and Hydrology was used for correlation analysis with litter quantity. A total of 13.8 metric tons (MT) of solid litter were removed from the Portoviejo River by the Azure system in 2021 and 2022, of which 6.2 MT (ca. 44%) was either confirmed by visual inspection to be plastic or estimated to be plastic based on the composition profile of domestic waste. Filled plastic bags had the highest contribution in weight for total waste removed and were related to domestic waste. Open dumping of domestic waste in plastic bags is a common practice and constitutes a main source of riverine contamination globally, and highlights the pressure on waste management systems in regions such as the Portoviejo River water basin. There was a significant negative correlation between litter quantity and precipitation, with more waste being collected during the dry season, which is in accordance with recent arguments that litter might have increased mobilisation in rivers only during extreme events such as strong floods. The data generated using the Azure system will be further used to validate riverine plastic emissions calculated using material flow analysis. Ultimately, the innovative technology involved in the Azure system represents a promising tool to inform policy measures with consistent data aiming to tackle local anthropogenic contamination.

#### **6.09.P-Mo516 Comparison of applicability of water column plastic sampling methods**

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Plastic pollution has been considered one of the major contemporary environmental challenges. Even though environmental effects associated with plastic pollution have been largely known, research on plastic concentrations mainly focuses on the marine environment. In recent years, an increasing number of studies reported environmental consequences and concentrations of plastic particles in freshwater systems comparable to those found in marine ecosystems. The magnitude of plastic particle abundance in ecosystems may be influenced by factors other than the real presence of plastics in the aquatic environment, such as sampling methods, and identification processes. Thus, a multitude of monitoring techniques have been used to collect information on the presence of macro- and mesoplastics in river systems. In this study, we assessed the variation in macro- and mesoplastic abundance and composition between two different sampling methods used to sample plastic pollution in the river Rhine, e.g., larvae net and trawl net. Additionally, we highlighted the strengths, weaknesses, opportunities, and threats (SWOT analysis) of the used methods for plastic monitoring. A difference was observed in the number of OSPAR categories in relation to the collected macro- and mesoplastic pieces between different methods. It was observed that the trawl net collects more different categories than the larvae net. However, the main categories follow the same patterns among methods, and the relative abundance per method slightly differs. The most dominant category in both methods was 'Plastic film 2.5 -50cm (soft)' and 'Plastic film 0 - 2.5cm (soft)'. A list of 20 factors was considered to obtain an extensive multi-criteria decision approach. The key factors can be grouped into monetary factors, for example, price per day, lab cost, and the minimum number of staff required; adaptability factors, for instance, if anchoring is required, different depths at which the method can be applied, the spatial location; operational factors, for instance, the width set-up from the boat and the net, the number of samples per day, the mesh size of the net; and limitations factors, for instance, the risk of the net getting clogged, permits required, the discharge sampling range, selective filtration limitations, and fish and plant bycatch. The outcome of the current study is relevant to assist policymakers in the development of targeted measures and mitigation strategies.

#### **6.09.P-Mo517 Establishing a methodology for monitoring microplastics in water intended for human consumption**

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Micro- and nanoplastics are ubiquitous pollutants with continuously increasing levels in the environment. As the awareness surrounding this issue continues to grow, so do the concerns regarding potential impacts on human health due to exposure to microplastics. Currently, evidences indicating any health effect are very limited. However, there is a need to better understand the possible risks. This requires a greater knowledge about our exposure through, for example, ingestion of food and drinking water. In order to gain more information regarding the exposure to microplastics, the recast Drinking Water Directive (DWD) of the European Union (Paragraph 6 of Article 13 of Directive (EU) 2020/2184) has empowered the Commission to adopt a methodology to measure microplastics in drinking water, which may then be placed on a watch list of pollutants that have to be monitored by Member States. As no internationally recognized standard method exists at this time, it has been necessary to undertake a targeted study to determine an appropriate methodology for the purpose of this legislation.

To support the establishment of a suitable methodology a literature review was carried out. The objectives were to identify the methods used to analyse microplastics in drinking water, including sampling procedures and analytical techniques used to identify and quantify microplastics. Capabilities and limitations of the published sampling and analytical techniques were reviewed and the quantities, size, composition and shape of microplastics found by published studies were examined. This was supplemented by consultations of experts in the field, nominated by the EU Member States. Elements of the suggested methodology – including sampling, various spectro-microscopic techniques and software solutions – were tested in the JRC laboratories.

The methodology suggested for application in the framework of the DWD foresees the use of in-line filtration as separation method and vibrational spectro-microscopic techniques ( $\mu$ Raman,  $\mu$ FT-IR, QCL microscopy) for identification and number-based quantification of microplastic particles water intended for human consumption. For monitoring purposes, the size range

of 20-5000 µm is targeted, and two groups of morphologies (“particle” and “fibre”) are considered, as well as the identification of priority polymers to facilitate consistent reporting.

#### **6.09.P-Mo518 The Unaccounted Presence of Plastic in Sea Turtles**

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Marine plastic litter poses a significant environmental threat in the 21st century, and while concerns are growing, available information on marine litter remains scattered and disconnected. This study focuses on the hypothesis that sea turtles, particularly species at risk, are profoundly affected by litter and microplastics. Current monitoring protocols, however, concentrate on plastics larger than 1 mm, neglecting smaller plastic sizes. Collaborating marine scientists from the University of Cadiz, specializing in marine litter, and veterinarians formed a team to investigate the impact of marine litter on two vulnerable sea turtle species: *Caretta caretta* and *Dermochelys coriacea*, prevalent in the Gulf of Cadiz and the Alboran Sea. Preliminary findings reveal a variable number of plastic debris items in all analyzed turtles, averaging 11 items per turtle, with an average plastic quantity of 32 mg. Plastic sizes varied from 0.2 to 127 mm, with a notable abundance of small fragments (0.5 to 15 mm), including identifiable items such as a gum wrapper, a lid, and a straw. The predominant plastic fragments extracted were composed of polyethylene and polystyrene. Despite these observations, the link between the presence of plastic items and their potential role as a cause of death, either indirectly or directly, remains uncertain and necessitates further investigation. Given the pervasive presence of plastic debris in sea turtles, particularly small-sized fragments, this study advocates for an updated and standardized methodology for identifying plastic debris in these organisms, proposing a lower size limit of at least 0.2 mm. Such standardization is essential for a more comprehensive understanding of the impact of plastic pollution on sea turtles and for the development of effective conservation measures.

#### **6.09.P-Mo519 Open Database of chemicals measured in plastic products**

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Plastics are widely used materials employed in various industrial sectors. They can contain various chemical substances, including residual monomers, additives, processing aids and non-intentionally added substances (NIAS), which can be released and negatively impact human and environmental health and the circular economy. Information on the presence and concentration of individual substances in specific plastic products is scarce and scattered. For this study we compiled the existing information by systematically reviewing recent measurement studies focusing on chemicals in plastic products. More than 4'000 substances have been detected in various products in recent literature. About 75% of these substances were only found in non-targeted workflows, often indicating non-intentional use. Targeted workflows and quantification efforts focused on a few typically well-regulated substances (e.g. metals, brominated flame retardants, *ortho*-phthalates). Overall, non-targeted studies show that many more substances than regularly measured in targeted analysis may be present in plastic products. At the same time, even when focusing solely on well-known and regulated substances, many new products on the market still contain them and thus can pose a risk to human health, the environment and the transition to a circular economy. Few studies have looked at the presence and concentrations in recycled plastics, and generally show large concentration variations but higher detection frequencies and concentrations in the recycled fraction. The compiled database may be useful for prioritizing substances, identifying blind spots and the need for additional measurements, and as input for substance flow analysis and exposure modelling to identify current and potential future exposure situations (e.g. when modeling circular economy scenarios). While the scientific community has made great efforts to monitor substances in plastic products, monitoring and subsequent removal are not sufficient to comprehensively manage hazardous substances in plastic products. In addition, cleaner production strategies and better supply chain communication are needed to ensure safe and sustainable use and recycling.

#### **6.09.P-Mo520 Vegetated Discharge Areas for Plastic Debris Removal: Deciphering of their Deposition on Various Substrates**

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Wastewater treatment plant effluent plays a significant role in the discharge of plastic debris into the oceans. Conventional treatment systems cannot allow for the retention of all microplastics (Carr et al., 2016). Developments known as vegetated discharge areas (VDAs) could improve this performance through terminal refinement. These zones made up of vegetated basins have several vocations, including filtering residual micropollutants (Boutin et Prost-Boucle, 2012). There is currently no data available on the capacity of these natural filters to retain plastic debris.

This work is a preliminary study, evaluating the performance of two substrates in retaining microplastics. An inorganic substrate conventionally used in ZRVs was tested, along with an organic substrate derived from a waste recycling process. Despite their recognized adsorption properties and nutrient-rich composition, organic substrates are rarely, if ever, used in ZRVs. For the sake of environmental representativeness, the microplastics used were obtained from plastic waste collected on a beach near the North Atlantic gyre. After mechanical fragmentation in the laboratory, microscopic analysis confirms that microplastics are polydispersed in size and polymorphic.

The experiments were carried out in columns, where a flow of microplastics passed through the substrates following two modes. Pulse injections are carried out to compare the substrates' retaining performances. Continuous injections are also performed to test the saturation of the substrates. Various parameters are tested, such as the volume of substrate in the column and the flow rate. In parallel, a reliable and robust method for quantifying microplastics is being developed using pyrolysis-gas chromatography-mass spectrometry. The substrates selected showed very high microplastic retention performance (70% on average), particularly for the organic substrate (up to 90%). The same experiments were carried out with potassium bromide as a non-reactive tracer, to further characterize the filtering properties of the substrates.

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#### **6.09.P-Mo521 Characterizing Micronized Plastic Particles (10-200 µm) for Exposure Studies and Risk Assessment – A Comparison of Different Methods**

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Microplastics (MPs) are widely recognized as multidimensional contaminants due to their variability in size, shape, polymer type, and chemical composition, all of which potentially impact their toxicity. Consequently, adopting a multidimensional risk framework for MPs that acknowledges the complexity of these contaminants is crucial.

Realistic exposure studies must reflect this multidimensionality. However, the challenge lies in preparing and characterizing micronized plastic particles that meet these criteria. To address this, we utilized four different plastic types (polypropylene (PP), polyethylene (PE), polystyrene (PS), and polyvinyl chloride (PVC)), that were artificially and environmentally aged, ground, and sieved to obtain a desired size fraction of 10-200 µm.

We analyzed the resulting particle suspensions of these micronized plastic particles utilizing four different techniques: Coulter Counter, FlowCam, LDIR chemical imaging, and stereomicroscopy. Preliminary results from FlowCam measurements indicate that the size distributions of the four different micronized plastic particles were similar in terms of the mean equivalent spherical diameter (ESD) and standard deviation. The smallest particles were observed for PS ( $17.99 \pm 11.21$  µm) followed by PVC ( $24.14 \pm 14.21$  µm) and PA ( $24.38 \pm 17.30$  µm), while the largest were amongst PP ( $31.61 \pm 17.57$  µm). Additionally, the predominant shapes of all the micronized plastic particles were quadrilateral and oval shapes.

When comparing concentrations in the size range of 10-120 µm the FlowCam results ranged from 2316 to 10464 particles per mL. In contrast, the results from the Coulter Counter were between 47 to 188 particles per mL, thus two orders of magnitude lower. However, both techniques agreed on PS being most abundant and PP being least abundant.

Further measurements using stereomicroscopy and LDIR chemical imaging will be conducted to investigate these differences and provide additional insights into particle size distribution and shape characterization.

The size, shape, and concentration of MPs are critical aspects in experimental laboratory studies and risk assessment. This study compared various techniques to provide particle counts and characteristics, enabling researchers to obtain comprehensive data on MP properties. Such data can aid in understanding the environmental impacts of MPs and address critical information gaps that hinder the ability to conduct reliable and relevant risk assessments.

#### **6.09.P-Mo522 The Application of Bayesian Networks to Integrate Microplastics and Nanoplastics into Regional Scale Multiple Stressor Risk Assessments: San Francisco Bay and the Delta as a Case Study**

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It is now understood that micro-, nanoplastics, and their leachates are part of the contaminant loading of marine, estuarine, and freshwater systems. These materials are only one of many other contaminants and other inputs that affect water quality. Current risk assessments for micro/nanoplastics are based on species sensitivity distributions (SSDs) derived from regressions of the results of toxicity tests to a variety of species. It has now been demonstrated that it is possible to build probabilistic Bayesian networks (BNs) to estimate risk to a variety of types of microplastics and to well understood contaminants (Hg, pesticides, organics EDCs) to provide comparisons to stressors undergoing regulation and management. Bayesian networks can incorporate specific pathways, be spatially explicit, incorporate other types of contaminants, include water quality parameters, and estimate risk to multiple endpoints derived from regulations and local stakeholders. San Francisco Bay serves as the model case study because of the extensive data base on chemical contaminants and the intense

sampling program conducted by the San Francisco Estuary Institute to quantify microplastics at that site. Toxicity tests demonstrate that there are multiple modes of action in addition to the dilution of or reduction to the quality of food uptake. The experimental data and the context provided by the BN relative risk model (BN-RRM) demonstrate the relative risk of each of these types of contaminants to the San Francisco Bay-Delta region. Uncertainty is explicit in the methodology and the relative assessment puts each into the current regulatory environment. The BN-RRM approach has a number of advantages in comparison to the SSD derived hazard quotients. First, the conceptual model is explicit with the cause-effect pathways clearly delineated. Second, the Bayesian network approach has been demonstrated in multiple previous environmental assessments including the effects of climate change. The various BN software tools allow for the characterization of uncertainty and sensitivity. Finally, BNs can be used to calculate the environmental required to meet the management goals. Consequently, microplastics and eventually nanoplastics can be integrated into the overall evaluation and management of contaminants for the study area and other aquatic sites.

#### **6.09.P-Mo523 Addressing Chemicals and Polymers of Concern in the UN Plastics Treaty**

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UNEP estimates that >13,000 chemicals, that is, additives, processing aids and non-intentionally added substances, are used or present in plastics. Being aware of the governance gap regarding these chemicals, many governments express the need to better regulate them in the global plastics treaty. However, the challenge to do so is substantial due to fragmented scientific knowledge and the sheer number of chemicals to consider. Therefore, we have built a harmonized database of >16,000 plastic chemicals and have developed a conceptual framework to identify and prioritize chemicals and polymers of concern. Here, we focus on our approach to regulate groups of chemicals as well as polymers of concern based on their content of hazardous chemicals. In an iterative process, we grouped >10,000 plastic chemicals in 80 groups based on their structures and prioritized groups based on the hazard profile of their members. We applied a similar approach for polymers: Using peer-reviewed, empirical data, we identified polymer types that have been shown to contain hazardous chemicals. For both aspects, we have developed multiple regulatory scenarios to predict the implications of certain policy decisions and support policymakers in taking decisions that are evidence-based and adaptive to the needs expressed in the plastics treaty.

#### **6.09.P-Mo525 CircleHealth – The Danish Hospital Sector Towards a Circular Transition of Textile and Plastic Waste**

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In 2020, the Danish Ministry of Climate, Energy, and Utilities, passed the Climate Act, committing Denmark to reduce its greenhouse gas emissions by 70 % (compared to 1990) and become climate neutral in 2025 (Climate Act, 2020). To achieve these national goals, four large mission-driven green research and innovation partnerships were established to accelerate the development of cutting-edge solutions with an equal focus on short-, mid-, and long-term impacts. One of these mission-based partnerships, focusing on plastics and textiles, is 'Trace – a transition to a circular economy'. This partnership facilitates collaborations between 90 partners including universities, knowledge institutions, and public and private companies, with a focus on the transition to circular economy (Kirchherr et al., 2017) for plastic and textiles (Trace, 2023). One of these projects is the four-year CircleHealth project, concentrating on textiles and plastics in the healthcare sector.

Extensive consumption of single-use medical products, materials, and resources in hospitals is often seen as a sterility necessity, resulting in massive amounts of hospital waste incinerated (Kane et al., 2018; Ramos et al., 2023). Despite being considered unfit for circulation, 85% of the general hospital waste is non-hazardous and can be compared to household waste (HCWH, 2020). Many of these products hold the potential for reduction, reuse, and recycling, and, thus, the CircleHealth project aims to explore measures to reduce plastics and textile consumption in Danish hospitals.

In CircleHealth, 11 partners - three universities, three Danish Regions, two knowledge institutions, and three private companies - collaborate to implement circular economy principles throughout the lifecycle of three selected plastic products and one textile product at partner hospitals. The project objectives take a starting point in the Waste Hierarchy as established in the EU waste framework directive (Directive 2008/98/EC, 2008), and aim to facilitate a vertical shift of the four healthcare products and move these, e.g., from 'incineration' to 'recycling', or from 'recycling' to 'reuse'.

The project's deliverables build on a mixed methods approach that includes the development of design strategies, Mass Flow Analyses and Life Cycle Assessments for the target products along with observations of consumption patterns and interviews with healthcare professionals to understand challenges and to test implementation.

#### **6.09.P-Mo526 From Teams® to Transects – Local Knowledge Challenges our Understanding of Beach Litter**

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In 1972, Gerald Scott published the seminal academic publication to link marine anthropogenic debris (hereafter litter) at sea to litter surveyed on two beaches on the Isle of Skye, a Hebridean island in Scotland, UK. Scott foreshadowed the state of marine and coastal environments today. We now know the consequences of this litter are varied, including animal entanglement and ingestion, chemical transport, and fragmentation. Despite representing ~32% of the UK's coastline, the Hebrides and northwest mainland Scotland are underrepresented in UK beach litter monitoring. But when surveys are conducted, beaches here represent some of the most polluted in the UK.

Here we present the findings of *50 Years of Litter on Skye*, a community co-designed research project that retraced Scott's footsteps with the help of the people of Skye. Two weeks of fieldwork were informed by six months of online community engagement. Nine beaches were surveyed following the OSPAR protocol, with survey areas split into 10m wide transects (the smallest width OSPAR permit). Where possible brand, provenance, and age were recorded. Community interviews were conducted to understand local beach cleaning efforts, common litter types, and the social significance of litter and beach cleaning in the community.

We categorised 13 909 items of litter across surveyed beaches, finding beach litter density to be one order of magnitude greater than the UK average. Litter populations varied significantly between beaches, with most litter associated with the fishing industry. Litter recovered dated back to 1989, and its provenance was traced to specific fishing regions along the east coast of North America. We found significant intra-beach variation between 10m transects, suggesting this survey width is not appropriate.

Community engagement identified specific issues with OSPAR categories. Adaptations to these may increase the suitability and uptake of OSPAR surveys in this region. Traversing the shore (Gaelic: *siubhal a' chladaich*) for marine debris has a long regional history, but the nature and value of the debris has changed considerably.

Accurately understanding beach litter is vital to informing industrial and legislative action. But standardising pollution survey methods for international applications necessitates generalisations that underrepresent, and fail to appropriately protect, certain coastlines and communities. Local knowledge is key in efforts to improve international litter monitoring.

#### **6.09.P-Mo527 Citizen Science Integration and Public Engagement for Plastic Litter Monitoring in the North Sea Region: Insights from the TREASURE Project in the Living Lab Nieuwpoort**

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Global plastic pollution is an ongoing and ubiquitous issue of environmental concern, requiring authorities, the industry, the public and researchers to work together and implement innovative methodologies to assess its impact and mitigate it. Within the framework of the TREASURE (Targeting the REDuction of pLAsTic oUtfLOW into the noRth sEA) project, we will initiate a series of targeted activities within the Living Lab of Nieuwpoort, Belgium. These activities will encompass mapping plastic accumulation and fate in the environment, identifying potential sources, and developing guidelines for effective collaboration with citizens and stakeholders, and to provide insight so mitigation measures can be established by policymakers based on our findings and results. Additionally, we will prototype a system to collect and remove plastic, and the knowledge gained will be shared and capitalized on for broader North Sea region improvements. To generate a broad dataset for plastic litter monitoring and to ideally spark environmental consciousness within the society, one of the strategies is to involve the general public in scientific projects, i.e. in citizen science projects. A successful example, among others, is the Plastic Pirates EU-project ([www.plastic-pirates.eu/en](http://www.plastic-pirates.eu/en)), in which high school teachers and children participate in litter collection observation campaigns on riverbanks, or near water bodies. We will apply the Plastic Pirates methodology in the Living Lab of Nieuwpoort (Belgium) and the data acquired from these campaigns will inform stakeholders about accumulation zones and types of litter requiring mitigation management actions. We will further focus on questions such as assessing the efficiency of citizen science observations of plastic litter and on how to improve the data flow, including for EMODnet submission, to inform local authorities. In this work we aim to demonstrate the integration of citizen science into sampling and monitoring strategies for plastic litter, providing valuable insights to empower and inform local stakeholders.

#### **6.09.P-Mo528 The PlastChem Project: Compiling material flows of plastic to prioritize action on polymers of concern**

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According to the Global Plastic Outlook report, the production of plastic has been increasing exponentially, from ~ 2 million tonnes/year in the 1950s to 460 million tonnes/year in 2019 (OECD, 2022). Moreover, the business-as-usual scenarios indicate

that plastic production would continue to expand from 460 to 1 230 million tonnes by 2060. Overall, about 2-19% of the plastic weight put-on-market consists of chemicals or additives such as plasticizers, fillers, antioxidants or flame retardants. Towards 2060, based on current trends, the mass of plastic chemicals is projected to increase by a factor of 5, from approximately 2-10 million tonnes/year in 2016 to 10 to 100 million tonnes by 2060. Of the plastics put on the global market each year, approximately 20% is landfilled and over 2% are emitted to the environment as micro- and macroplastics. Further, about 42% of plastic waste is incinerated, while 25% is recycled or reused. Both environmental emissions, landfilling and recycling present timepoints where both environment and humans can be exposed to hazardous plastic chemicals.

The PlastChem project provides a state-of-the-science report on plastic chemicals and polymers of concern that enables evidence-based policy development. Further, we aim to identify suitable measures, including suggested policy approaches for managing plastic chemicals and other actions that enable a non-toxic circular economy which ultimately reduces the spread of plastic chemicals and polymers of concern. With this purpose, material flow analyses (MFAs) of plastic and polymers were reviewed addressing the current and projected use and management of plastic in Norway, Europe, China and globally. Where available, we collected information from the different sectors “Packaging”, “Construction”, “Agriculture”, “Transportation”, “Electronics”, “Other plastics” which includes e.g. personal care and cosmetic products, household plastic and “Textiles” which includes technical textile, household textile and garments.

By analyzing the compiled MFAs, we have identified (i) sectors with high potential for improved waste management; (ii) polymers with high potential for recycling and reuse within each sector; and (iii) polymers of concern due to high potential for human or environmental exposure to their hazardous chemicals.

#### **6.09.P-Mo529 Sustainable Personal Care; Emission Estimation, Time-series Forecasting and Policy Measures for Microplastic Beads from Personal Care Products; A Study from India**

*Riya Kumbukattu Alex Miss, School of Environmental Studies, Cochin University of Science and Technology, India*

Plastic microbeads in personal care products are still an underestimated primary source of intentionally added microplastics. These are added for the purpose of cleansing, exfoliation and aesthetics. This study assessed 47 different categories of personal care products: face wash, face scrub, shower gel and body scrub from the Indian market and assessed their abundance, size, colour, shape and polymer composition. Also, an emission estimation and time-series-based forecast for 2030 were done using ARIMA (0,2,0) for individual categories and overall. The study reveals that 49 % of the products contained microplastic, with Polyethylene being the dominant one, followed by Polypropylene, Polystyrene, PMMA and Nylon 6,6. A significant number of products (22%) contained cellulose microbeads, with ambiguity regarding their biodegradability. The identified microbeads were mainly irregular in shape and granular in texture, especially scrubs. White/transparent colour dominated the identified microbeads, followed by blue, pink, yellow, red and green. The average size of microbeads was found to be 640.74µm, 452.45µm, 556.66µm and 606.30µm in different categories. The overall annual emission estimation was  $3.24 \times 10^{19}$  (highest),  $2.49 \times 10^{18}$  (lowest) and  $1.32 \times 10^{19}$  (average) in 2021 and forecasted to be  $3.82 \times 10^{19}$  (highest),  $2.95 \times 10^{18}$  (lowest) and  $1.55 \times 10^{19}$  (average) in 2030. This exorbitantly high value is due to India's significant population, globalisation, inefficient wastewater efficiency, growing economy and disposable income. A lack of policies and adequate regulations further add to this. The presence of other chemical like  $\text{TiO}_2$ , microcrystalline wax, etc need further studies to establish their potential harm to the environment and humankind. The presence of plastic exclusively from international brands which are also imported ones throw light into the ongoing transboundary movement of plastic. Thus, this study emphasises the need to develop stringent policies for India and other well-tailored mitigation measures. Some policy recommendations are also developed in this study to curb this threat.

Keywords - microplastics; emission; policy solutions; forecast model; personal care products

#### **6.09.P-Mo530 Event Ethnography to examine needs in the global negotiations on the Treaty to end plastic pollution: Dataset from the first session of negotiations (INC-1)**

*Emily Christine Cowan, SINTEF Ocean, Norway*

Plastics and their chemical pollution are now a planetary boundary threat. The recognition of this threat was demonstrated in March of 2022 when a historic mandate was adopted between United Nations Member States to begin negotiations on a treaty to end plastic pollution, taking a full lifecycle approach. The first round of negotiations began at the first International Negotiating Committee (INC-1) in Uruguay at the end of November 2022 and since then two additional sessions have taken place. In this presentation, Event Ethnography (EE) is utilized as a methodology to examine and document environmental agreement-making throughout the Global Plastic Treaty (GPT) negotiations. EE is a method increasingly recognized to study multilateral political and international negotiations within the science-policy nexus to examine emerging obstacles, trends, power dynamics, and actors (both state and non-state) at play in the negotiating arena. This research builds the foundation for systematically tracking the developments throughout the GPT negotiations via an ethnographic dataset. This dataset can be used to improve the understanding of the actors influencing negotiations, while also allowing scientists to understand the needs of policymakers within the negotiating arena while creating synergies with other Multi-lateral Environmental Agreements (MEA).

## 6.10 Science Communication: Reaching Outside of the Scientific Bubble

### 6.10.T-01 A Decade of Science Communication on Food Contact Chemicals: Approaches and Learnings

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Ensuring the public and its governing bodies have access to scientific research is pivotal for societal progress that is based on scientific facts. However, in the realm of science communication, effectively disseminating knowledge within and beyond the scientific community can be challenging. The intricate and nuanced nature of scientific information, particularly concerning environmental and human health issues, demands strategic communication methods. Even for experts within the scientific community, it is hard to identify and keep track of relevant publications amidst all the surrounding noise. Here we explore the complexities and approaches in bridging the gap between scientific research and diverse audiences.

We present a comprehensive framework developed over our years of experience in the field of science communication focusing on food contact chemicals and their interactions with human and environmental health. This includes presenting challenges faced, real examples from our previous communications, and some valuable insights gained along the way.

Our approach starts with the crucial aspect of adapting content for different audiences. Recognizing the diverse needs and levels of understanding between journalists, scientists, policymakers, or industry professionals, we supplement peer-reviewed publications with varied content forms. These include news articles, blog posts, press releases, illustrations, fact sheets, dossiers, and webinars. Each is based on a clear narrative and serves a unique purpose, catering to specific preferences and increasing the likelihood of engagement.

Furthermore, we offer tangible examples of successful (and less successful) science communication in action. We use a recent 2022 peer-reviewed article on migrating food contact chemicals as a case study. By employing a range of communication techniques, including developing narratives, visuals, and data-based tools, we effectively reached beyond the scientific 'bubble' into the realm of policymakers, advocacy organizations, and the concerned public.

Through sharing our science communication approach, we hope others can learn from our experiences and try out new communication formats to help them in expanding the reach of their own research.

### 6.10.T-02 Science Communication as a Tool to Recruit the Next Generation of Scientists

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Science and innovation are essential for solving the world's most pressing challenges. However, to attract the students to science requires multiple strategies, and especially effective communication strategies.

Over the past six years, we have actively engaged in initiatives and activities to attract students to our newly launched "Environment and Health" study program, mostly at the bachelor level. These efforts have included lectures and seminars at high schools, laboratory tours and workshops in our department, and also individual mentoring for high school students participating in scientific competitions. We have discovered that staying connected to the younger generation through social media is important, even though not all the social networks are used by teenagers; some are used more by their parents. Moreover, we have engaged the online influencer, who is known among young generation (especially high school students at the age 15 until 20), namely those who are interested in chemistry and STEM. Additionally, engaging high school teachers is essential as they play a significant role in informing students about study opportunities. To this end, we offer science programs for their classes and organize workshops for teachers focused on both science and teaching skills. Parents also exert a profound influence on their children's career aspirations, and we actively participate in public outreach activities such as Researcher's Night and the Festival of Science.

While some of our efforts have not yielded significant results, others have been successful. In this overview, we share what lessons have been learned from our science communication efforts, and what strategies are now fundamental aspects of our communication to general public and high school students.

### 6.10.T-03 Breaking Down Silos Between Science and Communication: U.S. Environmental Protection Agency's Innovative Approach to Research Outreach and Communications

*Esra Mutlu, Scarlett Vandyke, Jessica Daniel, Christina Baghdikian and Monica Linnenbrink, U.S. Environmental Protection Agency (US EPA)*

Scientists at the U.S. Environmental Protection Agency (EPA) conduct critical research to inform Agency decisions and support the emerging needs of partners and the public by advancing, developing, and applying innovative research to rapidly evaluate the health effects of chemicals and inform chemical risk assessments. Along with conducting cutting-edge science, effectively engaging end users of the science is vital to better understanding their research needs and increasing their awareness, understanding, and use of the science. Strategic stakeholder engagement and outreach assist with identifying



knowledge gaps and subsequent training opportunities and provide opportunities to collect and incorporate feedback into ongoing training efforts.

A common challenge in science is the ability to effectively communicate complex research to audiences representing various backgrounds, including non-scientists. Recognizing the importance of engaging with and training these audiences, EPA's research arm created an outreach, engagement, and training team to increase the awareness, understanding, acceptance, and use of new computational toxicology and exposure research data. A primary goal of the team is to establish scientific confidence in the application of new methodologies for regulatory decision making through a targeted New Approach Methods Training Program. The team uses a multifaceted approach, including user-friendly websites, informational and training programs, webinars, workshops, newsletters, social media, and pre- and post-feedback surveys.

This approach is already yielding positive results. Since its formation in 2022, the team has hosted 5 training webinars with nearly 500 average participants. Surveys of training attendees have shown increased intent to use tools as a result of trainings and overall satisfaction with the trainings and materials. The team's training website featuring tool resources has seen a 126.2% increase in users.

This presentation aims to provide an overview of the multifaceted outreach and engagement approach, results from engagement activities, examples of challenges encountered, outcomes of addressing these unique challenges, a summary on what went well and what didn't, and how lessons learned are being used to inform the future direction of these efforts.

The views expressed are those of the authors and do not necessarily reflect the views and policies of the U.S. EPA.

#### **6.10.T-04 What Are the Effects of Human Activity on Aquatic Ecosystems: Interdisciplinary Workshop and Hands on Training for Secondary School Students**

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Here we present a science communication initiative addressing environmental pollution's impact on aquatic organisms, particularly phytoplankton. The project targets secondary school students (13-15 years old) through an interdisciplinary teaching sequence (biology and geography) comprising 4-session workshop within build experimental kit. The sequence lets participants study the following guiding question: What are the effects of human activity on aquatic ecosystems? The interactive experimental kit simulates an aquatic environment with the green alga *Chlamydomonas reinhardtii*, showcasing its role in CO<sub>2</sub> consumption and O<sub>2</sub> production via photosynthesis. The kit's oxygen sensor tracks O<sub>2</sub> levels affected by pollutants from daily use products, demonstrating reduced photosynthetic activity and the interconnectedness between human activities and the environment. Pilot and main runs involving 72 and 123 students respectively evaluated the workshops via questionnaires and feedback, showing positive reception, especially regarding experiments and pollutant effects. Aligned with the French-speaking Swiss 10th/11th-year curriculum, these activities serve as a resource for educators interested in integrating environmental topics into science education. Furthermore, this initiative aligns with UNESCO's Global Education 2030 strategy, empowering learners with skills and values for a more sustainable society.

#### **6.10.P Science Communication: Reaching Outside of the Scientific Bubble**

##### **6.10.P-We537 Using a Consumer-App to inform citizens and pressure chemical substitution and regulation**

*Janna Kuhlmann and Luise Körner, Bund für Umwelt und Naturschutz BUND eV, Friends of the Earth Germany, Germany*

We know the story: Scientists find out, that a chemical substance poses a risk and then it takes forever, until it is regulated. Examples are microplastics, which recently have been regulated and PFAS, which are now in the process of being regulated. Often, a general awareness in the public and with policy makes is needed, before action is taken. Here, civil society organisations such as environmental NGOs play a crucial role to raise awareness on pressing issues.

Imagine, having an App, which informs consumers about the hazardous chemicals in their everyday products they buy and giving them the possibility to send complains.

Ten years ago, the non-profit environmental NGO BUND developed the ToxFox-App, which does exactly this. As for cosmetics the ingredients are known, we use scientific publications and lists of regulatory bodies to flag PFAS, Microplastics and Endocrine Disruptors, among others. We try to extend the information with new publications regularly. However, also toys, electronics and all kinds of other products can be scanned with the app to request data on substances of very high concern (SVHC) from the producers. According to the REACH regulation, consumers have this right to know. These requests increase pressure on companies to improve their chemicals management.

Regularly, a company takes a product from the market thanks to our work. However, we are relying on transparency on ingredients in products and on easily available scientific studies and lists of harmful substances and the products they are potentially used in for the success of this work.

Next to presenting the App and the challenges around it, the need for exchange between NGOs and scientists will be discussed. This includes open access to review articles and creating affordable places where civil society organizations can meet with scientists.

#### **6.10.P-We538 Bridging the Gap in Science Communication: From Scientific Understanding to Public Reception**

*David Mennekes and Denise M Mitrano, Environmental Systems Science, ETH Zurich, Switzerland*

Environmental pollution, particularly plastics pollution, is a topic of widespread concern among the general public. However, the pathway from new scientific understanding to become accurately presented information in media can be long and challenging. Moreover, when results are communicated, they are often given in isolated examples, but this approach often lacks general contextualization of how this fits into the broader picture of plastics pollution and impacts. We explored current constraints in science communication in the field of plastics research and what motivated journalists to report about plastics in the environment and under which conditions, were they writing about plastics. By surveying and interviewing a broad range of individuals working in science and media, we discerned how the communication of the risks of environmental plastics presented by scientists are perceived by journalists, public media, and ultimately the general public. To understand this, we focused on 1) how are scientific findings in the field of plastics research are communicated 2) how are science communicators in different media formats receive, filter and select this information and 3) how this information is translated into a digestible format suitable for the general public with different levels of interest, expertise and engagement with scientific topics. More specifically, we explored the communication of results by scientists, for instance, how the information of environmental concentration and environmental risks of plastics are perceived. Environmental concentrations are often presented either as mass concentrations (e.g., g plastics/kg soil) or particle concentrations (e.g., # plastics particles/kg soil); yet these contrasting presentations of environmental concentrations are not directly comparable and may lead to differences in comprehension of the extent of the issue. Ultimately, high particle concentrations may represent very low mass concentrations, but this information is not easily contextualized. Collectively this analysis on how to bridge the gap on environmental plastics pollution and risks communication can facilitate the interactions between journalists, and scientists, and consequently the general public will benefit from a streamlined, more digestible and targeted communication of scientific understanding.

#### **6.10.P-We539 Sharing your Science and Making it Sticky**

*Laura McConnell<sup>1</sup>, Timo Werneke<sup>2</sup>, Leah S Riter<sup>1</sup>, Lisa S. Ortego<sup>1</sup>, Greg Watson<sup>1</sup>, Zi Liu<sup>1</sup>, Sarah Eliza Lockwood<sup>1</sup>, Camille Ryan<sup>3</sup> and Carl-Christian Kolbe<sup>2</sup>, (1)Bayer Crop Science, (2)Bayer AG, Germany, (3)Bayer Crop Science, Canada*

Why do some scientific messages stay with you for days, weeks and even years later yet others are simply forgotten? Who are your science communication heroes? How do they deliver those memorable messages that inspire other scientists and the public? Scientists are trained in their technical disciplines and in the analysis of scientific data. They are trained to write proposals and peer-reviewed publications, but most scientists are not highly effective communicators. The quality of an engagement is determined by the quality of the connection you have created with your audience and not by the volume of data, evidence, and facts that you have shared with them. The moment we understand this is the moment we begin to truly engage. To quote Sydney J. Harris: "Information is giving out. Communication is getting through." Even within the scientific community, there are a diversity of audiences. It is important to know your listeners and consider their point of view. Too often we assume we know our audience, and don't care enough about their needs, or we use jargon and acronyms. There are tools that scientists can use to make their communications more "sticky" and impactful. Bayer carried out a "Science Engagers" training course for cohorts of scientists. These cohorts have continued to support each other after the training. One of the key tools in this training was focused on SHARP: Stories, Humor, Analogies, References and Quotes, and Pictures. This presentation will provide an overview of the SHARP tools and will provide some examples of different types of projects that emerged from participants to introduce these concepts to the SETAC community.

#### **6.10.P-We540 The Interweaving – of the synthetic and natural world**

*Hans Peter Arp<sup>1,2</sup> and Elizabeth Ellenwood<sup>3</sup>, (1)Norwegian University of Science & Technology (NTNU), Norway, (2)Norwegian Geotechnical Institute (NGI), Norway, (3)Artist*

Brought together by an interest and concern for pollution, an artist and scientist began having conversations and sharing resources. Through this collaboration, they discovered a common thread among their work:

The interweaving of the synthetic and natural world.

Together they created artistic images and science-based text expressed in a poetic form that are an honest depiction of what is seen and studied in research labs and the field. From microscopic images, collected objects from the sea, and written scientific studies. This poster offers insight into our current pollution challenges the natural world is facing. The viewer of the images and text are asked to question how they understand pollution, and what is their role in a sustainable future.

### 6.10.P-We541 ZeroPM on youtube

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The H2020 research and innovation project, ZeroPM, has an ambitious Youtube strategy. Over the course of our 5 year project we are targeting over 200 videos on the channel to disseminate and communicate the aims and outcomes of ZeroPM. To facilitate this, there were three main strategies: 1) develop an overall production element for all videos using standard vignettes and graphic templates, 2) different types of video content organized on channels and 3) assigned targets and deadlines videos for each partner to produce. The channels ZeroPM uses include: 1) Partner introductions: where each partner is tasked with introducing themselves, their facilities and what they will do in the project; 2) There is what where: used for content aimed at the general public, which are short, informative videos; 3) ZeroPM In depth: featuring technical interviews with ZeroPM partners; 4) ZeroPM Pieces: lunch time seminars given by members of ZeroPM to each other of ca 15-30 minutes of their research; 5) ZeroPM webinars: Webinars open to the public where a researcher in ZeroPM in collaboration with an external research give a broad 40 minute presentation of a research field with Q&A; 6) ZeroPM Science-Policy Webinars: where policy makers co-present with ZeroPM researchers on how the work effects the science-policy interface; 7) Workshop videos – videos filmed at workshops, including interviews and presentations; 8) ZeroPM news – where latest results of the project are presented as a news broadcast. Currently the most popular channels are related to the Webinar channels, followed by "ZeroPM-in depth", which includes music video "The PMT substances song".

Curious to learn more, visit our poster and <https://www.youtube.com/@ZeroPM-H2020>

### 6.10.P-We542 The PARC ambition to communicate to gain impact

*Sónia Namorado*<sup>1</sup>, *Marianne Bom*<sup>2</sup>, *Tamás Szigeti*<sup>3</sup>, *Maria Uhl*<sup>4</sup>, *Daniela Zanini-Freitag*<sup>4</sup>, *Dennis Sariannis*<sup>5</sup>, *Spyros Karakitsios*<sup>5</sup>, *Nikiforos Alygizakis*<sup>6</sup>, *Roser Gasol*<sup>2</sup>, *Dora Rolo*<sup>1</sup>, *Eugenia Dessipri*<sup>7</sup>, *Aglaia Koutsodimou*<sup>7</sup> and *Maria Silva*<sup>1</sup>, (1)National Institute of Health Doutor Ricardo Jorge, Portugal, (2)European Environment Agency, (3)National Center for Public Health and Pharmacy, Hungary, (4)Environment Agency Austria (EAA), Austria, (5)Aristotle University of Thessaloniki, Greece, (6)Environmental Institute, Slovakia, (7)General Chemical State Laboratory

The Partnership for the Assessment of Risks from Chemicals (PARC) is a public-public partnership using research and innovation for the benefit of human health and the environment by improving chemical risk assessment and supporting the implementation of new/better policies. More than 200 partners in 29 European countries are involved in the seven-year project co-funded by the European Commission (2022-2029).

In pursuit of its objective to significantly contribute to the EU Zero Pollution goal of achieving a non-toxic environment by 2050, PARC has an ambition to build bridges between academia, national and international regulatory bodies, industry, NGOs, decision makers and European citizens. The exchange of knowledge among these stakeholders, and potentially others, is crucial for PARC to gain real impact to protect human health and the environment.

Besides building interactions with Boards, including stakeholders (Stakeholder Forum) and experts from areas related to risk assessment (International Board), PARC developed a communication and dissemination strategy and is establishing synergies with external scientific activities, to boost its impact. The communication and dissemination strategy considered relevant methodologies for successful internal and external communication in order to reach specific target groups, e.g., industry, professional groups, and citizens, and engage them in supporting the aforementioned strategic goals. Various communication channels, including a website, four social media channels, and two newsletters were conceptualized and implemented. Further activities for engagement and exchange like a synergies network (SYNnet), facilitating collaborations between external activities and PARC, have been established to support the defined strategy. Local national hubs have also been established with the responsibility to build and strengthen local dialogues within each country and build the necessary connections with PARC.

In this presentation we will present the PARC ambition to gain impact, the organization of PARC and its communication and dissemination strategy, as well as the challenges PARC has been facing and is working to overcome in order to succeed.

### 6.10.P-We543 Communicating the Complexities of Plastic Pollution in a Polarized Media Landscape

*Nanna B. Hartmann*, *Environmental and Resource Engineering, Technical University of Denmark (DTU), Denmark*

In our endeavor as scientists to disseminate our findings, we are faced with a multifaceted challenge: Firstly, academia is a competitive environment and attracting funding is intertwined with our visibility and ability to communicate complex scientific ideas in an accessible manner. Secondly, visibility must be achieved in a saturated information landscape, which may tempt us towards oversimplified narratives to gain visibility. Thirdly, when operating within this space, maintaining our code of ethics and credibility as scientists in society is paramount. These challenges are accentuated within areas of broad societal awareness such as plastic pollution.

This presentation will draw upon personal experiences and lessons learned from interactions with the media under different circumstances. The focus, however, will be on a current podcast production: "Plast tur-retur" (Plastic roundtrip). The aim of this podcast series is to shed light on the causes of and solutions to plastic pollution, including biodegradable plastic as an

alternative material, sorting and recycling of plastic, as well as the need for legislation and changes in consumer behavior. This is achieved through conversations with fellow scientists, while transcending the boundaries of traditional academic discourse and reaching a broader audience.

In summary, this presentation will offer insights into the complexities of communicating scientific research on plastic pollution, striking a balance in the realm of visibility/credibility/responsibility. It will underscore the importance of upholding scientific integrity while effectively engaging with the public and media, thus contributing to a more informed and balanced understanding of plastic pollution and its interconnectedness with other environmental issues in society.

#### **6.10.P-We544 Phytoremediation for Public Engagement – An Interdisciplinary Project Bringing Together Art and Science**

*Jessica Chadwick, GEES, University of Birmingham, United Kingdom*

Soil pollution is a huge problem for the organisms that inhabit the soil, the people that use the land with wider nutrient cycling implications. Heavy metals, like lead, arsenic, and mercury, can have severe negative consequences on plant growth and development, and can be dangerous to human health. The soil contaminants found next to the Grand Union canal in Birmingham (UK) were many times greater than the recommended safety limits, with some found to be over 100 times greater than the safe concentration. Working together with local art and community project, Grand Union Gallery, we ran a community focussed project on site. We used a range of different plant species, including alder, sunflowers, and clover, to attempt phytoremediation of the area to try and reduce the mobility of these heavy metal contaminants, either through immobilisation in the soil or by uptake into plant tissues for safe removal. The project has now evolved into helping vulnerably housed people gain work experience, with individuals from across Birmingham getting involved in learning about soil science and pollution.

#### **6.10.P-We545 Scientific Communication from an Industrial Science Perspective: Gaps and Opportunities**

*David MV Saunders<sup>1</sup> and Erin Maloney<sup>2</sup>, (1)Shell Global Solutions, Netherlands, (2)Shell International, Netherlands*

Although professional courses in scientific communication are becoming more available and accessible, many students still undergo ‘informal training’ in science communication throughout the course of their studies. Often, this takes the form of developing posters or presentations for scientific conferences, reports for university or college classes, and/or publications to be submitted in scientific journals under the guidance of academic instructors or supervisors. Development of technical communication skills is very important for enabling peer-to-peer communication within the scientific field; thus, this type of (in)formal scientific communication training remains highly valuable for (under)graduate students. However, this type of training often overlooks aspects of scientific communication which are extremely important in the field of industrial science. Industrial scientists are often required to communicate with diverse stakeholders, regulators, and collaborators with differing backgrounds, perspectives, levels of technical expertise, and priorities. For example, along with peer-to-peer technical communication, industrial scientists may have to communicate with advocacy and policy experts, product stewardship, marketing and business representatives, industrial regulatory specialists, regulators, or manufacturers (amongst others). Thus, to ensure understanding, the same scientific output (e.g., idea/concept, study) will effectively have to be ‘translated’ for different stakeholders while retaining key concepts and a high level of informational accuracy. Moreover, this often occurs on a global scale, introducing additional challenges associated with of language, culture, and regional priorities.

This presentation aims to illuminate the unique challenges associated with scientific communication in industrial science, driving a discussion between the differences in academic and non-academic communication coming from the perspective of scientists working within industry. Altogether, we aim to highlight current gaps and opportunities to enable further development of tripartite scientific communication training practices in the SETAC community

#### **6.10.P-We547 Bridging the Gap Between Research and Stakeholder Needs for Effective Aquatic Pollutants Management**

*Katie Carter<sup>1</sup>, Maité Fournier<sup>2</sup>, Corinne Merly<sup>3</sup>, Nicole Baran<sup>3</sup>, Manon Berge<sup>2</sup>, Lara Bagheri<sup>1</sup>, Sabrina Giebner<sup>1</sup>, Gunnar Thorsén<sup>4</sup> and Thomas Track<sup>1</sup>, (1)Society for Chemical Engineering and Biotechnology e.V. (DECHEMA), Germany, (2)ACTeon Environment, (3)BRGM French Geological Survey, (4)IVL Swedish Research Institute*

Within the ERA-NET Cofund Aquatic Pollutants, there are 18 international research projects developing analysis, assessment and management solutions to address the presence of contaminants of emerging concern (CECs) and antimicrobial resistance (AMR) in water resources. Their exploitable results span products, technologies and materials, procedures and protocols, policy briefs, capacity building workshops, all addressing the presence and impact of these aquatic pollutants in various parts of the ecosystem chain. But what do the projects’ target stakeholders need to know, how does this align with the expected projects’ outputs, and how can these external stakeholders best be reached? These are the questions that the research within the Aquatic Pollutants TransNet (“TransNet”) transfer project is aiming to answer.

To answer this first question, we conducted over 50 stakeholder interviews from Germany, France, Sweden and the EU level, hosted two national workshops and analysed the national and EU regulatory context pertaining to CECs, AMR and pathogens. Through conversations with stakeholders from the public authority, water utility, applied research, NGO, industry, and research sectors, we established an inventory of ‘knowledge demands’, or knowledge needs stemming from the *insufficient*

*transfer of existing knowledge* or from *knowledge that is missing altogether*, classified by country, stakeholder sector and pollutant class (i.e. CECs, AMR). Coupling this inventory with an analysis of the state-of-art scientific knowledge and the expected outputs from the 18 research projects, we can align the knowledge demands of end-users to the research project outputs to support improved knowledge uptake and implementation.

This knowledge demands inventory will be developed into an online tool meant for researchers, providing a novel approach for connecting research outputs with stakeholder needs and aligning communication methods to stakeholder sectors – in this way answering our three initial questions. The communication methods and techniques will be developed for the various target sectors based on desk research, stakeholder interviews and results from various co-creation efforts. This tool will help bridge the gap between research and practical application in addressing aquatic pollutants – not only by assisting in dissemination activities, but also by identifying future research needs based on acute demands.

#### **6.10.P-We548 Sailing Scientists- Exploring water quality through water sports**

**Katie Reilly<sup>1</sup>** and **Iseult Lynch<sup>2</sup>**, (1)University of Birmingham, United Kingdom, (2)School of Geography, Earth and Environmental Sciences, University of Birmingham

Public engagement on environmental issues is increasingly acknowledged to be an important aspect of understanding and effectively addressing these challenges. Water quality has been increasingly reported as a concern to a range of different stakeholders, including dog walkers, anglers, water sports users (including open water swimmers), boat owners (such as canal boats) and artist/photographers.

Exploring water quality through the lens of water sports is an interesting and challenging angle, as most water sports users are increasingly concerned about the quality of the water that they are engaging with. This project works with Sea Cadets (UK) to assess water quality at their main boat stations, and to train a generation of young people (ages 9-18) in water quality assessments, experimental design and science communication throughout the project. Water quality assessments include pH, temperature, conductivity, nitrates and phosphates, dissolved oxygen, turbidity and visual indicators of pollution. Through these initial water quality parameters, we discussed areas of pollution that are of interest to the cadets and explored experimental designs to capture this going forwards, for example including plastic pollution and microplastic detection methods.

The ability for young people to have their voices heard is an important part of engaging the public in these topics. Ecoanxiety goes beyond focusing on climate issues to encompass a wider collection of environmental issues including water quality. Through the codesign process, the young people are supported and encouraged to investigate issues that are important to them, and given the opportunity to communicate these to different audiences. The results and outputs from this project are written into peer education training materials for use in groups ages 9-18, as legacy training resources.

In addition to working with a subsection of the public, this project also provided an opportunity to involve and support researchers with public engagement but providing a framework for training and experience. This enabled a diverse and engaging skills exchange between different groups which will hopefully inspire and support further public engagement projects and codesign research in the future.

#### **6.10.P-We549 Watch on the Rhine – RIWA-Rijn - Turning River Water Quality Data into a River of Quality Water Data**

**Gerard Stroomberg<sup>1</sup>** and **Rozemarijn Neefjes<sup>2</sup>**, (1)RIWA Rijn, Netherlands, (2)RIWA-Rijn, Netherlands

RIWA-Rijn is an association of drinking water companies in the Netherlands that use the river Rhine as a source. Established in 1952, making it one of the earliest NGOs worldwide to focus on protecting the river as an invaluable yet vulnerable resource for drinking water. In total 5 million people in the Netherlands depend on the river Rhine as their resource for drinking water.

RIWA-Rijn strives for such a quality of surface water that natural purification processes suffice to prepare impeccable drinking water. In line with Article 7 of the European Water Framework Directive, water quality must continuously improve, so that the level of the purification treatment effort can be reduced over time. RIWA-Rijn collects and disseminates data and knowledge on the water quality of the Rhine, informs governments and industry on our findings and represents the interest of its members in various (trans)national and European bodies. RIWA-Rijn maintains a database with water quality data of a vast number of substances, many of which go back over multiple decades.

RIWA-Rijn produces an annual report both in Dutch and German which describes the water quality of the Rhine in three parts. We assess the water quality of the Rhine on the basis of (1) the European River Memorandum that specifies a water quality that allows drinking water production with near natural treatment methods, (2) WFD article 7.3 which aims “to reduce the level of purification treatment required in the production of drinking water”, (3) the Rhine Ministers Conference target of a 30% reduction of emissions into the Rhine.

The data is collected from our own monitoring program, from our members and Rijkswaterstaat, the national Dutch water authority. Besides the targeted assessment we publish all our available data with general statistics such as annual averages,

minimum, maximum values and percentiles. And calculate 5-year trends for concentrations over 650 parameters, which include pesticides, pharmaceuticals, industrial contaminants, and consumer products.

Besides sending our report to in print stakeholders in the Rhine catchment, we make our reports available to the general public through our website. Monitoring downloads of our report show an increasing interest in our report, particularly in the Rhine catchment area. Other NGOs are increasing finding their way to our data and are using them for their own purposes, but most of all for protecting the water quality of the Rhine.

#### **6.10.P-We550 Full STEAM Ahead: Merging Science and Communications to Investigate Environmental Questions**

**Gretchen K. Bielmyer-Fraser<sup>1</sup>, Annmarie Kent-Willette<sup>1</sup> and James Nienow<sup>2</sup>, (1)Jacksonville University, (2)Biology, Valdosta State University**

This project focused on a case study and best practices surrounding a successful STEAM interdisciplinary research project. The study focused on graduate and undergraduate interdisciplinary research in the fields of Environmental Science and Communications. Specifically, a variety of water quality parameters and metals were measured, and phytoplankton abundance and diversity were assessed in water samples collected from eight sites in the lower St. Johns River, in Florida from 2019 to 2022. The project and resulting data were publicized using a multimedia communication approach including social media, in tandem with more typical scientific and academic approaches including presentations at meetings and journal publications. This project focused on how cross-discipline collaboration led to grant applications and ultimately secured funding, how the work successfully incorporated research opportunities for students, the role multimedia communications and a strategic social media plan played in community awareness and support, and how the project successfully communicated meaningful research while managing teaching across very different disciplines.

#### **6.11 Science for Global Management of Chemicals**

##### **6.11.T-01 Why we need science free of Conflict of Interest for Global Management of Chemicals**

**Miriam L. Diamond<sup>1</sup>, Andreas Schäffer<sup>2</sup>, Martin Scheringer<sup>3</sup>, Marlene Ågerstrand<sup>4</sup>, Ksenia J Groh, PhD<sup>5</sup>, Gabriel Sigmund<sup>6</sup>, Bethanie Carney Almroth<sup>7</sup>, Rainer Lohmann<sup>8</sup>, Anna Soehl<sup>9</sup>, Noriyuki Suzuki<sup>10</sup>, Marta Venier<sup>11</sup> and Penny Vlahos<sup>12</sup>, (1)University of Toronto, Department of Earth Sciences, Canada, (2)Institute for Environmental Research, RWTH Aachen University, Germany, (3)ETH Zurich, Switzerland, (4)Stockholm University, Sweden, (5)Eawag - Swiss Federal Institute of Aquatic Science and Technology, Switzerland, (6)EDGE, Wageningen University & Research (WUR), Netherlands, (7)University of Gothenburg, Sweden, (8)Graduate School of Oceanography, University of Rhode Island, (9)International Panel on Chemical Pollution, Switzerland, (10)National Institute for Environmental Studies (NIES), Japan, (11)Paul H. O'Neill School of Public and Environmental Affairs, Indiana University, (12)University of Connecticut**

The issue of Conflict of Interest (COI) is critical to consider when developing the structure and scope for the new Science-Policy Panel (SPP) for chemicals, waste and pollution prevention. Specifically, experts participating in the decision-making process and the core work of the SPP should not stand to gain materially from the work of the SPP or promote conflicting and/or incompatible outcomes or delayed implementation of solutions.

COI refers to financial or other related interests which could significantly impair an individual's objectivity or create an unfair advantage for any person or organization. It is unavoidable that every expert holds a particular point of view or perspective that could be seen as biased; here we focus on an individual, while pursuing a scientific question, would have a financial or material gain from a certain outcome of their scientific work, so that this gain is in conflict with the impartial investigation of the scientific question.

What are the tactics used by those having a COI? According to Schäffer et al. (2023), more than 20 tactics have been used by those with COI to counter scientific evidence or to promote narratives favourable to specific industry sectors. Examples are: Criticizing study designs or overemphasizing the shortcomings of scientific studies; Discrediting, intimidating or threatening scientists; Publishing misinformation; Cherry-picking data, designing studies to fail or come to a desired conclusion, or conducting meta-analyses that dilute scientific evidence; Extensive lobbying towards regulators and policymakers so that the voice of the vested interest is often the main or even the only one heard in public consultations.

For the work of the new SPP we recommend: 1. Define and strictly enforce rigorous COI provisions. Experts with a COI should not be allowed to participate in the decision-making process and the core work of the Science-Policy Panel, but may still participate and contribute as observers. 2. Implement independent audits to a) review compliance with the COI provisions, and, if needed, recommend corrective measures to the governing body, and b) ensure that the Science-Policy Panel's outputs are transparent, impartial, credible and scientifically robust, as mandated by the UNEA Resolution 5/8. 3. Include as many elements of transparency as possible and apply FAIR and CARE principles for scientific data management and stewardship.

##### **6.11.T-02 Sustainable Chemicals and Materials Policy – The Need to Develop Globally Binding Rules**

**Janna Kuhlmann<sup>1</sup>, Klaus Günter Steinhäuser<sup>2</sup> and Markus Große Ophoff<sup>2</sup>, (1)Bund für Umwelt und Naturschutz BUND eV, Friends of the Earth Germany, Germany, (2)Environmental Chemistry Working Group BUND eV, Friends of the Earth Germany**

Pollution by chemicals and waste is threatening human health and ecosystems on a global scale like climate change and biodiversity loss, resulting in UN framing it as a triple planetary crisis. Sustainable management of chemicals and materials deals not only with the assessment of hazards and risks of chemicals to health and environment in accordance with classic chemicals policy, but also the management of material flows from the extraction of raw materials up to waste. The constant increase in material flows threatens the Earth system. Persson et al. showed that the planetary boundaries for 'novel entities' have already been significantly exceeded. There exist close links between the increasing consumption of materials and the emission of climate gases as well as the losses of biodiversity.

A holistic sustainable chemistry can provide a significant contribution to the future management of substances, resources and materials. For chemicals, this means that they should be inherently safe, that is, they should not have hazardous properties. In particular, they should no longer be persistent. 'Forever chemicals' create problems that are ever increasing and irreversible. Material flows must be significantly reduced in order to reduce resource consumption significantly. Reuse and recycling of materials are crucial elements of a circular economy. The raw material feedstock of chemical production must also change: Alternative resources must replace mineral oil and natural gas. Three complementary strategies are critical to reducing material flows: efficiency, consistency and sufficiency.

Unlike climate change and biodiversity, no comprehensive legally binding international agreement exists for the management of substances and materials. Individual topics are covered, for example, in the Stockholm and Basel Conventions. The World Chemicals Conference in September 2023 resulted in a good declaration of intention. A trend to a legally binding framework convention is not discernible, however. One encouraging step may be the establishment of a Science Policy Panel on chemicals, waste and pollution prevention like the IPPC for climate and the IPBES for biodiversity. It will only be a success, if this committee will be independent and conflicts of interest will be avoided. The development of binding rules is urgent to avoid the overloading of the Earth system.

The work of environmental NGOs can benefit from an enhanced dialogue between science and civil society.

#### **6.11.T-03 The PlastChem Project: An evidence-based framework for identification and prioritization of chemicals of concern in plastic**

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Plastics are complex materials that contain a large number of diverse chemical substances, which can be released through the life cycle of plastics, i.e., production, use and end-of-life stages. They can negatively impact human and environmental health as well as hamper a safe and sustainable circular economy. Plastic chemicals are therefore one of the crucial aspects for the future Global Plastics Treaty. Yet, current information on plastic chemicals is fragmented and scattered over the public domain, and important gaps of knowledge remain on how to effectively and efficiently manage the large number of chemicals of concern in plastics. The PlastChem project aims to provide a state-of-the-science report on plastic chemicals and polymers of concern that enables evidence-based policy development. With this purpose, we systematically collected, compiled, and harmonized up-to-date scientific and regulatory information into a comprehensive database of all known plastic chemicals. We annotated these plastic chemicals with updated hazard information obtained from 15 regulatory and industry sources and conducted a weight-of-evidence analysis for the identification and prioritization of chemicals of concern, taking into account persistence (P), bioaccumulation (B), mobility (M), and toxicity (T). We further included additional regulatory-status information under existing chemicals and waste Multilateral Environment Agreements (MEAs) and scientific data on the presence and release of plastic chemicals from polymer plastics. We identified more than 16,000 plastic chemicals with unique chemical identities, from which more than 4,000 chemicals (25 %) were recognized as chemicals of concern following the assessment of their PBMT properties. Among the chemicals of concern, 13 % of them are currently being regulated under MEAs. Almost 4,000 chemicals were identified in extracts or leachates from plastics, and 25 % of them were among those identified chemicals of concern. Our weight-of-evidence framework integrates all this information, resulting in a compendium of lists that organize the universe of plastic chemicals according to different hazard scoring and prioritization levels. This work shed lights on an evidence-based regulation for identification and prioritization of chemicals of concern in plastic.

#### **6.11.T-04 POPs Global Monitoring, capacity building and data integration under the UNEP/GEF projects**

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The UNEP Chemicals and Health Branch, through Global Environment Facility funded POPs monitoring projects, data generation and capacity building in developing countries and countries with economies in transition, to facilitating the implementation of the Global Monitoring Plan (GMP) and evaluating the effectiveness of the Stockholm Convention (SC) on POPs. The second round of projects was implemented from 2016 to 2023, covering 42 countries. The key activities include monitoring POPs in core matrices air, water and Human milk, along with providing trainings and a global interlaboratory assessments. Data on POPs levels in humans and the environment are crucial for; ensuring the availability of data on exposure to POPs; understanding of data for effective actions in implementing the Convention; and strengthening global and regional

collaboration to address data gaps. Furthermore, capacity building is key component for generating high-quality data. Under the project, 26 training sessions have been conducted involving participants from 37 countries. The four rounds of interlaboratory assessments included results from 228 laboratories across 72 countries. Results showed that data gaps still exist, in particular for the newly listed POPs under the convention, as well as the growing complexity of chemical analyses.

The World Environment Situation Room (WESR) is UNEP's platform for data, information, and knowledge, facilitates access, visualization, sharing, and downloading of real-time information on the global environmental situation. The POPs monitoring dashboard, a complementary tool for data integration and sharing within the UNEP/GEF POPs projects, aims to enhance accessibility and understanding of data for a broader range of stakeholders, supporting informed decision-making alongside other data hubs like the GMP data warehouse. Long-term, high-quality data, information, and knowledge stand as fundamental pillars for assessments and decision-making processes. Establishing sustainable mechanisms to promote data usage in decision-making strengthens commitments to data generation and capacity building. Regional collaboration plays an important role in bridging data gaps. Mechanisms should be in place to enable contributions from broader stakeholders, to support data generation and the sound management of POPs. The knowledge generated from POPs is critical for addressing other global challenges such as plastics, climate change and biodiversity loss.

#### **6.11.T-05 Assessing Regional / National Capacity for Monitoring and Research on POPs in Water in Selected UNEP regions**

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Knowledge of water concentrations of persistent organic pollutants (POPs) is important for understanding sources and global distribution as well as exposure of aquatic organisms and their food webs. The Global Monitoring Plan (GMP) under the Stockholm Convention (SC) on POPs currently includes data for perfluorinated alkyl substances (PFAS), but not for other POPs in water (<https://www.pops.int/Portals/0/download.aspx?d=UNEP-POPS-COP.11-INF-38.English.pdf>). The goal of this study was to assess the extent of the measurement data for monitoring of POPs in surface waters of Africa, GRULAC (the UNEP region comprising Latin America and the Caribbean), and Asia-Pacific, as well as adjacent coastal seas and oceans. With this information we assessed national capacities for measurement of POPs in water and propose a strategy for the monitoring at the national or subregional level. A detailed review was conducted of scientific papers and technical reports on chemicals listed under the SC that had been measured in surface waters over the time period of 2011 to 2022. The DDT and HCH groups predominated with over 150 reports in freshwater for 4,4'-DDE, 4,4'-DDT and  $\gamma$ -HCH. Endosulfan, dieldrin, endrin, aldrin, heptachlor, and heptachlor epoxide were also frequently reported. The results of this review identified laboratory and institutional capacity, expertise, data availability, and existing interlab programs that could form the basis of future monitoring of POPs in water. While no formal global monitoring program exists for POPs in water, the recently completed AQUAGAPS-MONET passive sampling study has provided a limited picture for non-polar POPs in global surface waters (Lohmann et al. ES&T 2023). The latter project used a central lab distribute passive samplers and to analyse samples. The use of a central expert lab for global studies of POPs is generally recommended, however, it does not provide for capacity building at the national and regional level. Taking the above points into consideration, a future framework for water sampling and POPs analysis at the national and subregional level would ideally be based on passive samplers provided by a central lab but analysed by individual labs. This strategy has already been used for interlab comparisons of passive samplers. The results would contribute to the GMP but would provide information on concentrations in water at the national level that could be useful for broader water quality assessment goals.

#### **6.11.P Science for Global Management of Chemicals**

##### **6.11.P-Mo531 Recommendations for the Deliverables of the new Science and Policy Panel on Chemicals, Waste and Pollution Prevention**

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The United Nations Environment Assembly (UNEA) resolved to establish an independent, intergovernmental science-policy panel on chemicals, waste and pollution prevention (SPP). In accordance with resolution 5/8 an Open-Ended Working Group (OEWG) was established with its charge resolve anticipated for June 2024. Following efforts to set priorities and define the work of the SPP we present options for deliverables that may aid in the framing of the upcoming SPP's charge. We compare reports from both the Intergovernmental Panel on Climate Change (IPCC) and the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) and identify areas in which deliverables of the SPP can complement and provide synergistic advances to existing reports. We propose possible frameworks for SPP deliverables while preventing overlap and duplication of efforts. Consequently, we also propose possible reports that are likely to arise from member states and how these too may compliment current IPCC and IPBES efforts.



### 6.11.P-Mo532 The International Panel on Chemical Pollution (IPCP): Academic scientists' voice in support of the Intergovernmental Science–Policy Panel on Chemicals and Waste

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In 2020, the IPCP called for the establishment of an intergovernmental **Science–Policy Panel (SPP) on Chemicals and Waste** to address gaps in current science-policy interface in international chemicals and waste governance, including the lack of (1) coverage relative to the large and growing universe of chemicals and waste; (2) horizon scanning and early warning mechanisms; (3) bidirectional communication between policy-makers about the scientific community, and (4) engagement of the wider scientific community. International negotiations on such a panel are currently on-going.<sup>1</sup>

In 2023, key principles for the SPP were published<sup>2</sup>, in particular (1) to fill a critical gap pertaining to the mounting and accelerating impacts on human and environmental health caused by chemical pollution and waste globally; (2) to avoid “paralysis by analysis” by repeatedly re-assessing the same topics and substances; (3) for a broad and inclusive SPP to properly respond to the breadth and complexity of global chemical production, use, releases, and disposal; (4) to be policy-relevant but not policy-prescriptive to support sound, evidence-based policy development; (5) Governments should nominate independent experts as the main “workforce” of the SPP, with independent scientists providing primary scientific results; (6) to establish and enforce a strict conflict-of-interest (COI) policy; (7) include a wide range of data from the peer-reviewed scientific literature, gray literature, and existing biological and chemical monitoring programs; (8) to apply a transdisciplinary approach to the integration of knowledge, across different temporal and spatial scales and different regions of the world; (9) for the outcomes to be policy-relevant, but not policy-prescriptive; and (10) to ensure that all countries are adequately represented in terms of experts and their backgrounds, data, and access to information.

The importance of a strict COI policy for the SPP is needed to avoid established tactics to manufacture doubt in favor of vested interests.<sup>3</sup> The authors rebutted an assertion voiced by some that the chemical industry should be directly involved in the panel's work because it possesses data on chemicals essential for the panel's activities. Steps should be taken to prevent the detrimental impacts of conflicts of interests on the panel. In particular, an independent auditor should be included in the panel to ensure that participation and processes follow clear COI rules.

### 6.11.P-Mo533 Obstacles to Scientific Input in Global Policy

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The planetary crisis, as identified by the United Nations, stems from climate change, biodiversity loss, and pollution. Addressing these challenges requires informed decision-making based on reliable scientific insights. However, the utilization of pertinent research in policy planning is hindered by constraints imposed on scientists' involvement in the process by policy-making entities. While the United Nations Environment Programme (UNEP) encourages the engagement of independent scientists without conflicts of interest, the accreditation process poses difficulties for many due to stringent eligibility criteria. UNEP mandates that accredited organizations cannot receive government funding, creating challenges for scientists affiliated with government-funded institutions, including universities.

This restriction, while aiming to avoid conflicts of interest arising from political messaging, fails to differentiate between government-funded institutions with scientific independence, such as universities, and those that may compromise scientific integrity. As a result, public universities, in Europe and globally, face exclusion from UNEP accreditation. Scientists from government-funded institutions may seek alternative entry routes, like joining national delegations or non-governmental organizations (NGOs). However, such pathways may compromise their independence and raise concerns about the credibility of their policy recommendations.

A viable alternative for scientists affiliated with government-funded institutions is accreditation through multilateral environmental agreements like the Basel, Rotterdam, and Stockholm (BRS) Conventions. This underutilized option offers a

less stringent process, increasing the likelihood of eligibility. We argue that UNEP should enhance the visibility and accessibility of this alternative. Furthermore, we propose that UNEP, with the backing of member states, should recognize universities as independent, self-governed entities eligible for direct accreditation. This shift, coupled with support from academic institutions, would empower scientists to contribute effectively to global negotiating processes. By improving accessibility and inclusivity, UNEP can ensure the meaningful participation of independent scientists in policy negotiations, leveraging their trusted collective knowledge for a more sustainable future.

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#### **6.11.P-Mo534 Enhance the Science-Policy Panel's Impact by Leveraging Synergies with Innovation**

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With UNEA Resolution 4/8, sustainable chemistry has been anchored as being an element of the sound management of chemicals and waste. Furthermore, the United Nations Environment Programme's Green and Sustainable Chemistry Framework Manual highlights the crucial importance of environmentally sound innovation. Innovation implementing new chemistry-based concepts, products, processes and services should therefore be perceived as a pathway towards sustainable chemistry solutions and the prevention of pollution. This should also be reflected in the remit for a corresponding science-policy panel.

In order to evaluate the importance of sustainable chemistry innovation in the aforementioned context, the ISC<sub>3</sub> database of 210 start-ups from all over the world has been analysed and a thorough sustainability assessment of the proposed innovation and business concepts has been conducted. The corresponding solutions are predominately motivated by solving immanent (local or regional) problems and challenges. They are characterised by a holistic life-cycle thinking avoiding environmental trade-offs and often show a strong socio-economic innovation component, creating employment for local communities and facilitating capacity building at regional level in conformity with the Agenda 2030. 60% of the entrepreneurs are situated in the global south including African and Latin American countries or India. Likewise the annual ISC<sub>3</sub> innovation challenges show a strong participation from the global south, indicating that innovative sustainable chemistry solutions are not biased towards the global north. Entrepreneurship activities and their success thereby strongly benefit from supportive framework conditions, including policy recognition and incentivisation, visibility and facilitation of investments. The SPP could promote and facilitate innovations and contribute to visibility. Overall, awareness about approaches to and merits of innovations based on sustainable chemistry can pave the way to policy-relevant and impact-oriented recommendations of the future SPP.

#### **6.11.P-Mo536 Monitoring of Toxicity of Plastic Recyclates from Low- and Middle Income Countries by Bioassay Panel to Support the Global Management of Chemicals in Plastics**

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More than 13,000 chemicals are associated with plastics including more than 3200 chemicals with hazardous properties. The large number of non-intentionally added substances (NIAS) including degradation products, processing aids, impurities in plastic additives and transformation products are present to various degree and are often not monitored by instrumental target analysis. In particular the recycling of plastic result in complex chemical mixture which even can include POPs if e.g. e-waste plastic is recycled into toys. There is a lack of knowledge of POPs and other hazardous chemicals in plastic recyclates and related toxic risk in particular in low- and middle income countries (LMICs) which often lack analytical capacity. Therefore, activities were conducted under the UNEP/GEF Global Monitoring Plan projects to monitor plastic recyclates from Africa, Asia, and the GRULAC region. In addition to target POPs analysis also the screening of toxic effects of selected plastic recyclates can contribute valuable information to expose risk. We applied a panel of human cell-based biological detection methods (i.e. CALUX assay) to assess the migration from plastic recyclates used to produce food or skin contact products on a range of toxicity pathways (e.g. cytotoxicity, genotoxicity, oxidative stress, endocrine effects, and PAH toxicity). The migration experiments were conducted by comparing three different extraction/migration experiments (A: THF/hexane; B: 50% ethanol/water at 60°C for 3 days; C: 20% ethanol/water at 40°C for one day). Highest in vitro toxicities were found in PVC LDPE, and HDPE recyclates from Nigeria. Cell death, PAH-, estrogen- and anti-androgen-like toxicities were frequently detected. None of the initial samples tested showed genotoxic effect (no p53 DNA repair activation by p53 CALUX). In most cases the full extraction Method A resulted in considerable higher toxicity compared to the two migration tests (B and C). Such effect-based bioanalysis allows for detection of the integrated toxicity of the chemical mixture in plastic recyclates and can be used in combination with instrumental analysis for effect-directed analysis. Since bioassays require relative low resources and maintenance of equipment while covering known and yet unknown toxic relevant compounds, they could be a useful tool for monitoring and controlling toxic chemical mixtures in plastic recycling in LMICs.

#### **6.11.P-Mo537 Enhancing POPs Measurements and Reporting for Air: Keeping up with the Demands of the Global Monitoring Plan**

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This paper summarizes the findings related to air, for a UNEP report on assessing POPs measurement capacity in air and water and strategies for sustainable data generation to support the Global Monitoring Plan (GMP) of the Stockholm Convention (SC) on POPs. The SC on POPs is an international agreement that aims to protect human health and the environment from the harmful effects of POPs. Global monitoring data for the listed POPs in core media (i.e., air, human milk and blood, water) is compiled and reported every 6 years in support of the Effectiveness Evaluation (EE), which informs the Conference of the Parties (COP) on the state of progress and relevant issues with respect to the listed POPs. A re-occurring issue in past GMP reports is the challenge for monitoring programs to keep pace with an expanding list of POPs in particular in developing countries and countries with economies in transition, which has more than doubled (since 2004) to 34 POPs.

A secondary aspect of this paper is to highlight some of the new developments under the Global Atmospheric Passive Sampling (GAPS) Network - also related to the topic of enhancing POPs measurements in air and meeting future challenges through cross-cutting studies. As the only continuously monitoring global-scale program investigating POPs in air, the GAPS Network is raising awareness and generating impacts through cross-cutting research at the science-policy and policy-policy interfaces. Examples will be presented on new areas of GAPS-driven research on topics such as, *inter alia*, health, climate, biodiversity and chemical waste.

#### **6.11.P-Mo538 Do simple screening criteria capture a chemical's potential for adverse effects in remote regions?**

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The Stockholm Convention (SC) on Persistent Organic Pollutants (POPs) provides a framework for global action on substances which are likely to lead to significant adverse environmental and/or human health effects in remote regions as a result of long-range environmental transport (LRET). However, in order for risk profiles on nominated chemicals to be prepared and evaluated (Annex E), the chemicals first need to be deemed to fulfill four screening criteria outlined in Annex D (persistence, bio-accumulation, LRET, as well as adverse effects). We used a modified version of the OECD P<sub>OV</sub> and LRTP (long-range transport potential) Screening Tool ("The Tool") for assessing chemicals for persistence (P) and LRTP to analyze (i) if all screening criteria (Annex D) really need to be fulfilled for a chemical to be likely to result in adverse effects as a results of LRET and (ii) to what extent the existing modelling approach recommended by the OECD is therefore fit for the purpose of identifying chemicals proceeding to the risk profile stage. Based on a model-based analysis of 12,615 organic high production volume chemicals (HPVs), we find that a set of alternative LRTP metrics, referred to as the Emission Fractions Approach (EFA), classifies a larger number of HPVs as having the potential for accumulation in surface media of remote regions than is classified as POP-like by the existing method recommended by the OECD. Many chemicals in the screening data set with the potential to accumulate in surface media of remote regions do not meet the screening criteria for LRET (half-life in air < 2 days) and P (half-life in water < 2 months) of the SC. We conclude that screening for LRTP under Annex D using the existing OECD Tool could potentially prevent chemicals from proceeding to the risk profile stage or being nominated in the first place. It is therefore recommended that assessments of a chemical's potential for accumulation in surface media of a remote region, such as offered by the EFA, should play a more prominent role in model-based assessment under Annexes D and E of the SC.

#### **6.11.P-Mo539 Needs for Sound Science across Existing National and International Management Structures – Implications from Observation in a Global Mercury Study**

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Variety of national and international organizations have been working at the management of chemicals. At least partly because of the real wide diversities of chemicals in their nature, and also the long history of chemicals management as probably one of oldest environmental concern on pollution, each country established their original legal/national frameworks and also several international organizations have worked on the issue from each standpoint, of course with efforts of collaboration among them [e.g., 1].

Now many chemicals are used in all global, nearly ubiquitous manner. Chemicals that will be long-range transported in natural environment are managed by specific legally-binding instruments like Stockholm Convention on POPs, however, many chemicals that may be outside the scope of such global frameworks also may exist in the environment ubiquitous way, maybe because of widespread and common use in the world, transport by artificial mechanisms, or maybe other reasons. Clearly as we should not establish independent global legally-binding instruments for all hazardous chemicals for each, we need to think about better structure of chemicals management in global system.

The discussion here is based on the outcomes of a research project "Intervention Scenarios and Global Mercury Modelling for Effectiveness Evaluation of the Minamata Convention on Mercury" [2], which was lead by a co-author Takaoka and participated by another co-author Suzuki in this abstract.

As observations and conclusions based on our case study on mercury, the holistic understandings of chemicals in globe is essential and effective for global management, however for many chemicals and waste issues, including industrial chemicals, e.g., PFAS, plastic additives, widely-used pesticides and other examples, may not have such sound scientific understandings. To achieve the most trustful and sound scientific understandings, we need independent scientific structure consisting of wide range of sciences in the way of avoiding any conflicts to the interest that expecting analysis may refer.

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### **6.11.P-Mo540 Needs for sound science across existing national and international management structures – implications for the marine ecosystems**

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The chemical production of new compounds has followed an exponential increase between 1800 and the present, with an average annual rate of increase of 4.4%. In 1800, about 40 manufactured compounds were in use and in 2023 more than one million [1]. Specific legally binding instruments exist, such as the Stockholm and Minamata conventions on POPs and mercury, respectively, but it is clear that the high number of new chemical species introduced into the environment and into the human diet necessitates an IPCPW that can address this enormous challenge in a holistic manner [2]. Poorly managed waste is one of the priority pathways by which these compounds enter the environment. Marine ecosystems receive enormous quantities of these chemicals, such as pharmaceuticals, personal care products, petroleum residues, flame retardants, pesticides, plastic additives, prefluoroalkyl and polyfluoroalkyl substances, nanoparticles, microplastics and nanoplastics, to name a few. Untreated or poorly treated wastewater is one of the main carriers of these pollutants. These waters are usually discharged in the areas of influence of the coastline, i.e. the marine zones of greatest productivity and biodiversity.

The number of countries that treat more than 75% of wastewater discharges is very limited, which implies a regular discharge of pollutants into the marine environment, especially in coastal areas, where biodiversity is higher and a substantial part of fish and seafood is collected for human consumption. These discharges may also be mediated by rivers, for example, an estimated 275 million metric tons of plastic waste were generated in 192 coastal countries in 2010, of which between 4.8 and 12.7 million metric tons entered the ocean [3]. In addition, maritime areas have eventually been used as waste dumps. Ship traffic is also source of pollutants if they release untreated sewage or ballast waters into the oceans and seas, particularly near harbour areas while waiting to be loaded or unloaded. In addition, some compounds such as mercury and halogenated persistent organic pollutants can be incorporated into the oceans through long-range atmospheric transport.

An important aspect to elucidate about these discharges is the residence time of the contaminants, which in the case of microplastic debris has been calculated to be much longer than previously expected [4]. This finding implies a generalization at source of the presence of contaminants in the marine system and the need to consider the waste released in a global context rather than relating it to local discharges.

According to the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, one in five people worldwide depend on wild species for food and income, with fisheries being one of the main sources of food from wild species. Unfortunately, many of the pollutants discharged, especially those with high chemical and environmental stability, return to humans because they accumulate in fish, as is well known in the case of mercury and microplastics.

The future IPCPW should focuss specific attention to the management of discharges of sewage and waste in the marine areas. This approach will allow to creating synergies with the promoters of panels specifically devoted to the marine system such as the International Panel on Ocean Sustainability [5].

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### **6.11.P-Mo541 A Cheminformatic Exploration of Chemicals on the Global Market**

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The years since the Second World War have seen exponential increases in the volume and diversity of chemicals produced, but a failure of regulatory capacity to keep up. Consequently, although chemical pollution has been recognized as one of three planetary crises threatening the survival of humanity, many chemicals in commerce remain poorly understood and studied.

Building off previous work by Wang et al. (2020), we compile an updated global inventory of chemicals in commerce, covering more than 350,000 chemical entities across 23 regulatory jurisdictions, and including specialized inventories such as the European Inventory of Cosmetic Ingredients. By applying cheminformatic tools to screen these chemicals, we gain the most in-depth understanding of the properties and diversity of chemicals on the global market.

In particular, we use fingerprint-based clustering approaches to assess geographic and temporal trends in chemical usage, identifying chemical families common to multiple inventories, or those unique to certain jurisdictions. We also use data on the registration dates of chemicals to identify areas of chemical innovation. In parallel, we investigate more specific questions, including whether the more than 100,000 chemicals listed as previously registered in the European Union and which were not carried over into REACH ceased production or were instead listed in other jurisdictions.

To assess the extent to which computational models for property prediction are valid for chemicals on the global market, we identify chemicals falling outside or on the borders of the applicability domains for several quantitative structure-activity relationships, and make practical recommendations to facilitate improved assessment of these chemicals. For those chemicals that do fall within the applicability domain, we make several property predictions to explore the property distributions of chemicals on the global market.

It remains a challenge to obtain unambiguous chemical identifiers for all chemicals on the global market – only 70% have their assigned CAS numbers identified, and UVCBs, polymers, and mixtures account for 21% of the inventory. However, our analysis provides important insights into trends in the chemical industry and regulatory efforts, as well as targeted recommendations for improving regulatory capacity to assess novel chemicals.

#### **6.11.P-Mo542 Continuing large-scale global trade and illegal trade of highly hazardous chemicals**

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Anthropogenic chemical pollution stands as a significant contributor to the interconnected triple planetary crisis of climate change, biodiversity loss and pollution. One important instrument in regulating the cross-border movement of hazardous chemicals is the Rotterdam Convention, providing nations with a framework to manage the international trade of specified perilous substances. However, an evaluation of the Convention's effectiveness has been notably limited in existing research. In our comprehensive analysis, we delved into 66,156 trade records sourced from the United Nations Comtrade database. This rigorous examination revealed that, between 2004 and 2019, a staggering 64.5 megatonnes of 46 listed chemicals were traded. Alarming, illegal trade was pervasive, accounting for at least 25,324 trade records and a volume of 25.7 megatonnes. While the Convention demonstrated positive impacts for over 70% of the listed chemicals, persistent large-scale trade of notorious substances like tetraethyl and tetramethyl lead persists. Addressing these concerns necessitates concerted global efforts. Improving the global trade dynamics of highly hazardous chemicals requires enhanced enforcement of the Rotterdam Convention, tackling illicit trade head-on, and promptly including problematic chemicals—such as chrysotile asbestos—within the Convention's regulatory purview. Such concerted global action is imperative to safeguarding the planet from the detrimental consequences of unregulated chemical in commerce.

#### **6.11.P-Mo543 How Do We Address the Global Threat of Irreversibly, Accumulating Trichloroacetic Acid (TFA)?**

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Trifluoroacetic acid (TFA) is a small, hydrophilic, non-degradable molecule that has been detected in diverse environmental media, from precipitation and ice cores, air, soils and sediments to human serum and plant-based foods and drinks. TFA, which is also regarded as the smallest of the per- and polyfluoroalkyl substances (PFAS), has been referred to as a “substance from multiple sources (SMS)”. TFA was not commonly reported in non-atmospheric environmental media until very recently. All of these reports collectively indicate its concentration is orders of magnitude higher than other PFAS.

One major source, particularly responsible for the accumulation of TFA in remote areas suand in precipitation are “F-gases”, a group of hydrofluorocarbons used as part of the Montreal Protocol's substitution from the ozone-depleting chlorofluorocarbons (CFCs). Some emerging F-gases allowed by the Montreal Protocol, such as HFC-1234yf, are anticipated to accelerate the irreversible, global accumulation of TFA, by orders of magnitude from 2050, depending on future developments of the global regulations, including the Montreal Protocol. However, even if F-gases are banned, there are many other sources, like pesticides, pharmaceuticals and industrial substances containing a -CF<sub>3</sub> group, or (fluoro)polymers. TFA concentrations will increase in the coming decades and it is unclear when and if it will ultimately plateau.

The global threat of TFA is that while it is increasing it may cross a threshold concentration that will cause severe ecological harm. Eco(toxicity) data is scarce, as most conclusions are based on aquatic studies that are near two decades old. In these studies, microalgae are considered the most sensitive ones. Current reported eco(toxicity) thresholds, e.g., PNECs for

freshwater are reported to span between 0.12 µg/L and 5.6 mg/L. For terrestrial plants, where TFA accumulates in very high concentrations, safety thresholds based on toxicity tests corresponding to these high concentrations have not been determined yet.

Here, we will demonstrate the “case” of TFA as an omnipresent, persistent, and mobile contaminant that presents a planetary boundary threat. The TFA emitted today will be a legacy for countless of generations in the future. Global policy action towards solutions will be presented that will enable the plateauing of TFA concentrations, to prevent its planetary boundary from being irreversibly exceeded.

#### **6.11.P-Mo544 Geospatial Dependent Pesticides Application Pattern for Selected European Countries**

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The overall volume and quantity of pesticides currently employed for agricultural purposes in the European Union (EU) are rising due to demographic growth and increasing demand for food since the most efficient way of boosting agricultural yield is through utilization of plant protection chemicals. While the pesticides usage is inevitable, their occurrence and influence on the environment have attracted the attention of scientists and regulators due to several imperatives, such as regulatory compliance, optimizing application practices, and selection of safer pesticides. Nonetheless, the first step to environmental studies is foundational comprehension of the input of pesticides into the environment. To estimate the input of the pesticides into the environment, it is essential to have information about the applied pesticides, their corresponding application quantity, and the targeted crops for each region. To achieve this objective, the integration and combination of two databases were undertaken for selected EU countries: 1) a registration database, detailing plant protection products registered for use, describing the approved quantity and pesticide composition for diverse crop types; and 2) a crop database, presenting geospatial information on cultivated crops. The registration database for each country was being harmonized in terms of layout, resolution, and language. The harmonized data were integrated and new datasets containing the information about the pesticides generalized application pattern for each country were generated. The new generated datasets were connected to the crop database and the integrated data were transformed into the maps illustrating generalized application pattern for each region. The outcomes of this investigation furnish valuable insights into the geospatially dependent potential input of pesticides into the environment, offering substantial utility in subsequent exposure, environmental fate, risk assessment studies and in general, essential for management of pesticides use.

#### **6.11.P-Mo545 Can We Track the Origin of Rapidly Rising Releases of Hexachlorobutadiene to the Global Environment?**

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Hexachlorobuta-1,3-diene (HCBD) has been listed in Annex D and E of the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2015 and 2017, respectively, meaning that both deliberate and unintentional production should be curtailed globally. Yet, air-monitoring has revealed that HCBD concentrations have been rising rapidly and continuously from 2008 to 2021, making it the most prevalent POP in the global atmosphere. Despite a long atmospheric residence time, HCBD's air concentrations are far from uniform in the Northern Hemispheric, but show a strong spatial gradient: Whereas background concentrations in the Northern Hemisphere increased from ~100 pg m<sup>-3</sup> to ~1000 pg m<sup>-3</sup> during the 2010s, levels recorded across Japan are somewhat higher (1,000 to 4,000 pg m<sup>-3</sup>) and concentrations in Northeastern China, even distant from suspected sources, exceed 100,000 pg m<sup>-3</sup>, often reaching levels over 1,000,000 pg m<sup>-3</sup>. The unintentional production of HCBD in China has been estimated to have risen from ~100 MT a<sup>-1</sup> in 2000 to 3000 MT a<sup>-1</sup> in 2016, mostly as a result of tri- and perchloroethylene (TCE, PCE) production. We are using time-variant simulations with the BETR-Global model to explore whether the estimated unintentional Chinese emissions can explain the temporal and spatial trends observed in the atmosphere or whether additional emissions need to be invoked. We further discuss the implications of the apparent failure of the provisions of Article 5 of the Stockholm Convention in reducing, let alone eliminating, the environmental releases of unintentionally produced POPs, especially considering that the unintentional production of HCBD during TCE and PCE manufacturing has been known for many decades.

#### **6.11.P-Mo546 Synthesis of Radiolabelled Industrial Chemicals, Drugs and Crop Protection Products from Carbon-14 Building Blocks**

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Carbon-14 (<sup>14</sup>C) is the key radioactive isotope used in metabolism studies supporting the development of industrial chemicals, drugs and crop protection products. Carbon-14 used in radiolabelling studies across the world originates from [<sup>14</sup>C]carbon dioxide which is typically stored as [<sup>14</sup>C]barium carbonate. This molecule is considered as the universal starting material from which all other carbon-14 labelled compounds are prepared. Four core building blocks stem from this universal starting material; [<sup>14</sup>C]carbon dioxide, [<sup>14</sup>C]metal cyanides, [<sup>14</sup>C]acetylene and [<sup>14</sup>C]barium cyanamide.

The four core building blocks can be further expanded via a variety of different pathways to give a multitude of different families of low molecular weight <sup>14</sup>C labelled intermediates. Access to these low molecular weight <sup>14</sup>C labelled building blocks allows for complete control when designing and developing the radiolabelling of a test item. As a result, multiple synthetic

routes can be designed and multiple radiolabelling sites within the target molecule proposed. This process ensures a one-stop-shop, entirely controlled radiosynthetic process from design to execution to single or multiply radiolabelled test items.

The poster will show how these pathways can be utilized to provide options for the radiolabelling of industrial chemicals, drugs and crop protection products.

#### **6.11.P-Mo547 Glyphosate and earthworms**

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Introduction: Reliable data on the toxicity of this herbicide, not only to earthworms, but to soil invertebrates in general, are scarce and not consistent, leading to a lack of clarity on this subject. Aiming to shed light to the topic, we decided to review the literature using an exhaustive research to answer two questions: 1) Which endpoint is the most sensitive when assessing the effects of glyphosate or GBH in earthworms? 2) Are glyphosate and GBH harmful to earthworms when used at the recommended application dose (RAD)?

Materials and Methods: keywords in ISI Web of Knowledge and Web of Science, using the “All Databases” option, with the following formula: ‘*earthworm\* OR lumbric\* OR aporrect\* OR eisen\* OR dendrob\* OR alloloboph\* OR octolas\* OR dendrodril\* OR diporodril\* OR eophil\* OR helodril\* OR kritodril\* OR octodril\* OR prosellodril\* OR scherothec\* OR satchell\* OR proctodril\* OR orodril\* OR postandril\* OR perelia\* OR andrei\* OR fetid\* OR foetid\** AND ‘glyphosate or roundup or Round-up or glyphosate-based-herbicide\*’ in Topic.

Results and Discussion: Out of 307 potential papers, only 63 papers could be used, indicating that around 80% of the papers initially found on our search did not have sufficient and relevant information. The low number of papers assessing toxicity to reproduction (19% of all papers) can lead to an underestimation of the toxicity of the herbicide to this endpoint. Most of the papers using one application of the recommended application dose (RAD) did not find any impact of glyphosate or GBH on earthworm survival. However, glyphosate or GHbs, under RAD can cause toxicity to cocoon production, lysosomal destabilisation biomarkers (neutral red retention time or NRRT), number of juveniles, and others.

Conclusions: The review showed that glyphosate and GHB can be toxic to earthworms. Although different factors can influence toxicity and much remains unknown, data points towards negative impacts on reproduction and the provision of ecosystem services. Our results also indicate that the risk assessment process has gaps and is not strong enough to prevent future threats to biodiversity. There is an urgent need for government agencies to keep pace with updates on competent directives and other concerning legislation, standardised methodologies, and the development of the field of soil ecotoxicology, aiming to avoid possible underestimation of the potential risk of a pesticide in the near future.

#### **6.11.P-Mo548 Conflicts of interest in scientific publishing: a cross sector perspective**

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Scientific publishing is an essential part of the scientific process, allowing researchers to disseminate their findings and contribute to the advancement of science. However, conflicts of interest (COI) can arise when personal views or financial incentives have the potential to bias results in scientific publications. While financial COI are often easy to recognize and already disclosed in the relevant sections of the publication (e.g. the private sector), authors from other sectors such as universities, non-governmental organizations (NGOs) and regulatory agencies often explicitly state in their papers that COI do not apply to them. We argue that while not necessarily financial in nature, relevant COI exist in all research sectors. In academia for example, they include the career-defining necessity to publish in high-impact journals and dependencies on grants, which are exemplified by integrity scandals including large scale falsification of research results. In contrast, NGOs often pursue a societal or political agenda, which can create COI independent of financial interests. While the nature of the COIs can be sector-specific, we argue that all stakeholders, regardless of their affiliation, need to clearly identify their sector specific COI and explicitly disclose their COI in their publications to allow for a more objective judgment of the presented work. We would like to use the SETAC EU meeting in Seville to invite scientists from all sectors to contribute ideas, suggestions, opinions and potentially co-author an envisioned short publication.

#### **6.12 Science-Policy Dialogue on Per- And Polyfluoroalkyl Substances (PFASs) Towards a PFAS-Free Future: Latest Development and Future Needs**

##### **6.12.T-01 European Regulation of Per- and Polyfluoroalkyl Substances (PFASs) and its Connection to Research**

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Risks from Per- and Polyfluoroalkyl substances (PFASs) are currently not adequately controlled. Therefore, in January 2023 the authorities from Denmark, Germany, the Netherlands, Norway and Sweden submitted a joint proposal to restrict all PFASs under Regulation (EC) No. 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals

(REACH), based on the shared concern about their very strong persistence. The proposal for a restriction of the non-essential uses of presumably more than 10,000 PFASs is one of the most comprehensive restriction proposals put forward thus far.

In order to understand the discussion around this broad PFASs restriction proposal and also to know where and how to best contribute with scientific data and knowledge, it is important to understand the restriction procedure, how scientific insights were used in the past and the future course of the broad PFASs restriction proposal.

The presentation is mainly based on the work of the following restriction proposals and preceding or follow-up work:

- Identification of PFOA as a Substance of Very High Concern (SVHC) under REACH and the restriction proposal following this identification
- Broad restriction proposal for PFASs under REACH.

The aim of the presentation is to show how scientific data and knowledge have contributed to the PFASs regulation under REACH from past to present and which research areas will be of relevance from a regulatory point of view in the future. It will also elaborate on the complex process of preparing the broad PFASs restriction proposal and outline how a step-by-step phase-out of PFASs in the EU through the implementation of the restriction could look like.

Due to the high relevance of scientific data and knowledge within regulatory processes discussions to continuously maintain and improve the link between science and regulation are of very high importance. The presentation is intended as contribution to facilitate this discussion

#### **6.12.T-02 Database of Alternatives to Per- and Polyfluoroalkyl Substances Based on the Functional Substitution Approach**

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Per- and polyfluoroalkyl substances (PFAS) are a group of fluorinated organic substances. The typical properties of the perfluoroalkyl moiety in PFASs (e.g., thermal and chemical stability, combined oleophobicity and hydrophobicity) have resulted in extensive use across diverse industrial and consumer applications. Due to their extreme persistency in the environment, regulatory activities have been increasing over the last few years to phase-out all uses of PFAS which encourages companies to find PFAS-free alternatives for their uses of PFAS. The aim of this study is to propose a database which (1) describes the uses of PFAS in order to understand the purpose they serve in each of their application; and (2) gathers information on potential alternatives which could deliver similar services. The main purpose is to gather information on alternatives to PFAS and to make it more accessible so it can be used to guide companies in their substitution activities. Following the functional substitution approach, the database provides information on the technical functions, end-uses and services provided by PFAS application of PFAS. Potential alternatives are also listed along with a short description of any potential loss in performance compared to PFAS, and whether they are available on the market or not. The annexes to the background document to the REACH restriction proposal on uses of PFAS served as the main source of information to build the database. In total, 37 different technical functions of PFAS were identified, which corresponds to up to 201 different services across 261 applications. The results suggest that waterproofing agent, corrosion inhibitor and heat stabiliser are the most common technical functions delivered by PFAS. It also suggests that PFAS deliver more than one service in the majority of the applications which makes the search for alternatives more challenging. Further analysis is still needed to identify the cases where PFAS could be phased-out because they do not provide a technical which is critical for the performance of the application. As of November 2023, 563 different alternatives are listed in the database for 211 applications. The majority of the alternatives identified are alternative substances or alternative materials. Further analysis is still needed to determine the cases where PFAS can already be substituted as suitable alternatives are already available and in use.

#### **6.12.T-03 The PFAS Analytic Tools: Using Geospatial Information to Promote Transparency in Understanding and Managing Risk from Per- and Poly-fluoroalkyl Substances in the United States**

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Per- and poly-fluoroalkyl substances (PFAS) are recognized as an urgent public health and environmental threat across the global community. In response, the United States Environmental Protection Agency (U.S. EPA) has developed a PFAS Strategic Roadmap documenting commitments to action in three domains: (1) research to increase understanding of PFAS risk and risk management responses; (2) restriction to prevent PFAS from further entering the environment at harmful levels; and (3) and remediation of existing PFAS contamination to protect human health and the environment. A key element needed to implement this strategy is transparent integration of the rapidly growing body of information about the species, presence, transport, fate, and potential exposures of PFAS in the environment. To address this, U.S. EPA has developed the PFAS Analytic Tools, a public web-based geographical information system for integrating data about PFAS reporting, testing, and occurrences in communities across the United States.



The PFAS Analytic Tools aggregates state and national PFAS data from 11 different datasets into a single searchable online platform. Users can filter data by PFAS or geographic area, explore application charts and maps, and download data for further exploration in other tools. The system integrates multimedia data from multiple federal and state agencies and is designed to be updated continuously as new data become available. The PFAS Analytic Tools enables risk managers, researchers, and community stakeholders to better understand potential PFAS sources in their communities and to develop effective management, policy, and other actionable responses.

This presentation will discuss the design of the tool as well as present several examples of applications to inform policy and management of risk from PFAS, to promote communication and transparency amongst communities and stakeholders, and to help address environmental justice concerns.

#### **6.12.T-04 Per- and polyfluoroalkyl substance (PFAS) immobilization approaches with regenerated waste products in large scale unsaturated lysimeters**

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There are estimated to be more than 2,000 sites in Europe in which soil and leachate water contain highly elevated concentrations (>100 ng/L) of per- and polyfluoroalkyl substances (PFAS). The accumulative emissions of PFAS from these sites are a threat to groundwater and drinking water reservoirs. Immobilization technologies, aiming to stabilize PFAS within the soil and thereby prevent PFAS leaching, are a promising in-situ soil remediation strategy. However, a detailed understanding of the transport of PFAS in the unsaturated zone after soil amendment is currently lacking. Since fossil based PFAS binders (e.g. activated carbon from anthracite) are associated with high carbon footprints, there has recently been an emerging interest for biochar-based binders derived from waste, which can be produced with net negative carbon emissions. The presented study used 10 outdoor lysimeters to investigate how the presence of commercially available soil amendments and such biochars could be used to stabilize the transport and fate of PFAS in the unsaturated zone and to lower bioavailability of these substances.

The lysimeters ( $n = 10$ ), with the exception of one lysimeter serving as a clean blank, were spiked with an Aqueous Film Forming Foam (AFFF) and a mixture of 18 PFAS. Two lysimeters remained non-amended, while two lysimeters were treated with commercially available PFAS immobilization binders (PAC, RemBind<sup>®</sup>). The remaining four lysimeters were amended with two types of biochar in two different concentrations each, one tested biochar being pyrolyzed from timber waste and a second one from PFAS-contaminated sewage sludge. Leachate water samples were collected weekly when available. After ending the experiment section-wise soil samples were taken from each lysimeter. All water and soil samples were extracted and analyzed for target PFAS. At selected time points the leachate was analyzed for dissolved organic carbon (DOC), anions, cations and heavy metals.

This study provides novel insight on how soil stabilization techniques, especially biochars can be used to reduce leaching of PFAS from highly legacy contaminated sites. Sludge derived biochar showed promising results in lowering leachate of PFAS from contaminated soil and can be a more sustainable and greener solution for soil remediation than traditional sorbents. Moreover, the potential to derive biochar from PFAS-contaminated feedstock represents a crucial step to handle PFAS contaminated organic material.

#### **6.12.T-05 Insights Into the Atmospheric Sources and Fate of (Ultra-) Short-Chain Perfluoroalkyl Carboxylic Acids from New High Time Resolution Measurements**

*Cora Young<sup>1</sup>, Jessica Clouthier<sup>1</sup>, Shira Joudan<sup>2</sup>, RenXi Ye<sup>3</sup>, Trevor VandenBoer<sup>3</sup>, Eric Vanhauwaert<sup>4</sup>, Jeremy Wentzell<sup>5</sup>, Mayre Rodriguez<sup>1</sup>, John Liggio<sup>5</sup> and Na-Yung Seoh<sup>1</sup>, (1)York University, Canada, (2)Chemistry, University of Alberta, Canada, (3)Chemistry, York University, Canada, (4)Department of Chemistry, York University, Canada, (5)Environment and Climate Change Canada, Canada*

The atmosphere is well-established as an important source and transport pathway for persistent perfluoroalkyl carboxylic acids (PFCAs). Despite its importance, measurement techniques for the gas and particle phase are limited to time-integrated methods, which limit our understanding of atmospheric processes. We have developed two in situ measurement techniques for PFCAs that allow us to capture PFCAs in the gas and particle phases. These techniques operate at timescales (i.e., seconds to one hour) that are similar to those of the atmospheric processes we wish to understand. We used the acetate chemical ionization mass spectrometer (CIMS) and ambient ion monitor-ion chromatograph-mass spectrometer (AIM-IC-MS) to make ambient atmospheric measurements of C2-C6 PFCAs in Toronto, Canada. These measurements clearly demonstrated the importance of PFCA atmospheric formation. Diurnal profiles of gaseous PFCAs were consistent with photochemical formation, with significant correlations to other photochemical products, such as ozone and formic acid. Particulate PFCAs were observed by AIM-IC-MS and showed a relationship to overall particle loading. The implications of these new observations for PFCA atmospheric fate and transport will be discussed.

## 6.12.P Science-Policy Dialogue on Per- And Polyfluoroalkyl Substances (PFASs) Towards a PFAS-Free Future: Latest Development and Future Needs

### 6.12.P-Tu531 Understanding Perfluoropolyethers and Their Life Cycle

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Per- and polyfluoroalkyl substances (PFASs) comprise a class of synthetic chemicals that have attracted much public attention since the early 2000s. Over time, research and risk management measures have expanded to a wide range of PFASs, but mostly on non-polymeric PFASs. To ensure the sound management of the entire class of PFASs, it is equally important to understand polymeric PFASs, which include side-chain fluorinated polymers (SCFPs), fluoropolymers and perfluoropolyethers (PFPEs). This poster presents a synthesis report by the OECD/UNEP Global PFC Group, synthesizing scientific and technical information on the life cycle of PFPEs, which are polymers that contain perfluorinated ether moieties such as  $-\text{CF}_2-\text{O}-\text{CF}_2-$  as repeating units direct in the polymer backbone chain. It starts with a comprehensive overview on the chemical identities of PFPEs that have been on the global market, including a non-exhaustive list of 153 PFPEs. 134 of them have their assigned Chemical Abstracts Services Registry Numbers (CASRN) identified, and only 23 have structures readily available on SciFinder. Then, the life-cycle information of different PFPEs is synthesized, including the production and use of respective PFPEs, presence of other PFASs in the commercial formulations, degradation of PFPEs during use and end-of-life treatment, and environmental releases of PFPEs and other PFASs present in the commercial formulations. Compared to many non-polymeric PFASs and other polymeric PFASs such as SCFPs, less public information is available on the life cycle of PFPEs. Nevertheless, a variety of structurally diverse PFPEs exist on the global market, and their use in various industrial applications and consumer products is extensive. Noticeably, many PFPEs have rather low molecular weight, even below 1000 Da in some cases. Some evidence does show that other PFAS may be present in PFPE commercial formulations from different origins, but further investigation is needed to capture a comprehensive overview. PFPEs are generally considered to be stable and inert, with high thermal stability. However, they are vulnerable to Lewis acid-catalyzed decomposition, including during some uses. These findings are only a first step towards understanding the life cycle of PFPEs and the extent of their use on the global market. Concerted action by all stakeholders is needed to address PFPEs in an efficient and effective manner, building on the gaps identified in the report.

### 6.12.P-Tu532 Emission inventory of PFAS and other fluorinated organic substances for the fluoropolymer production industry in Europe

*Joost Dalmijn, Department of Environmental Science (ACES), Stockholm University, Sweden*

Fluoropolymers are part of the broad class of substances known as per- and polyfluoroalkyl substances (PFAS). During their production, a wide array of additional fluorinated organic substances (many PFAS and some not defined as PFAS) are used, formed and emitted to air and water. This study aims to assess, and make an inventory of, all emissions of PFAS and other fluorinated organic substances by the fluoropolymer production industry in Europe using available emission databases and permits. Air emissions of the fluorinated gases chlorofluorocarbons, hydrofluorocarbons, hydrochlorofluorocarbons and perfluorocarbons (CFCs, H(C)FCs and PFCs) by this industry have reportedly decreased between 2007-2021 from roughly 500 to 150 tonnes/year. Emissions of fluorosurfactants to air and water have also been reduced significantly. However, large uncertainties remain regarding the emissions of substances that are neither CFCs, H(C)FCs, PFCs nor fluorosurfactants but are classified as PFAS, such as polymerization by-products, chain transfer agents and fluorinated solvents. The available data indicate that the release of these substances is not decreasing but remains relatively stable. As the current inventory probably underestimates emissions, further research, improved data availability and more harmonized reporting of emissions are necessary to obtain more accurate emission data for these substances. Nevertheless, based on the available data, it is clear that the emissions from fluoropolymer production plants to air and water are still significant and that the production of fluoropolymers continues to introduce persistent substances to the environment.

### 6.12.P-Tu533 Are Analysis-of-Alternative Methods Suitable for Per- and Polyfluoroalkyl Substances (PFAS) under REACH?

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As society transitions away from per- and polyfluoroalkyl substances (PFAS), it is crucial to avoid regrettable substitution and prevent the lock-in of undesirable substances in future technologies. Existing methods for analysing chemical alternatives, such as GreenScreen® and Multi-Criteria Decision Analysis (MCDA), have been recommended by regulatory bodies, but their suitability for the European marketplace and for PFAS and their alternatives remains unclear. This study evaluates seven analysis-of-alternatives (AoA) methods, focusing on their compatibility with PFAS and REACH. GreenScreen® and MCDA are further investigated using hypothetical and real substance datasets. Results reveal disparities between GreenScreen® and REACH, emphasizing the need for caution in interpreting GreenScreen® results, particularly regarding hazards like mobility and persistence. MCDA parameters are found to significantly influence outcomes, necessitating careful selection for substances with highly heterogeneous hazard profiles. A modified MCDA approach is proposed for analysis of chemical alternatives, including mixed aggregation, non-linear value functions, and an objective hierarchy with dependant attributes. The study underscores the importance of informed method selection when deciding on alternatives to PFAS with emphasis on limitations on the current version of GreenScreen® and the sensitivity of MCDA to parameter choices. The research's impact

lies in enhancing the effectiveness of AoA methods for PFAS alternatives under REACH, helping optimise the transition from PFAS, and contributing to the broader objective of a toxic free society.

#### **6.12.P-Tu534 Exploring the Presence of Per- And Polyfluoroalkyl Substances (PFAS) in Dutch Surface Waters**

*Sanne Brekelmans, The Water Laboratory, Netherlands*

Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic chemicals that are ubiquitous in the aquatic environment. Exposure to PFAS is often associated with many adverse health impacts, including effects on the immune system and development of the unborn child<sup>1</sup>. Their unique physicochemical properties can make PFAS very persistent while also being water soluble. As a consequence, PFAS are poorly removed by conventional treatment steps and are partially discharged in surface water (SW). In the Netherlands, approximately 40% of drinking water (DW) is produced from SW. Therefore, PFAS are of concern for the preparation of DW.

The Water Laboratory developed an ultra-high sensitivity analysis of PFAS<sup>2</sup>. This method achieved sub-parts per trillion detection levels for 26 PFAS compounds, with LOQ values of down to 0.2 ng/L<sup>2</sup>. The method included perfluorocarboxylic acids (PFCA) and perfluorosulfonic acids (PFSA) and other PFAS such as HFPO-DA (Gen-X) and precursors. Additionally, the method separates and semi-quantifies *branched* isomers of PFOA, PFOS and PFHxS. Trifluoroacetic acid (TFA) is additionally monitored in a separate method.

Here, we show the highlights of our extensive PFAS monitoring data from different Dutch SW. The data demonstrate consistent presence of PFCAs, PFSAs and moderate frequency of other PFAS. The shortest-chain PFAS, TFA, was the dominant congener detected at µg/L level, whereas longer-chain PFAS were detected at the ng/L level. Branched PFAS were also frequently detected at concentration levels in the same order-of-magnitude as linear PFAS, thereby appearing as relevant to SW as their linear twins.

From 2026, PFAS levels will be limited in the EU at 100 ng/L for 20 specified PFAS, for which the investigated SW would pass<sup>3,4</sup>. The EU also proposes a 500 ng/L limit for a 'Total PFAS' parameter, that is, the totality of all PFAS -including the mentioned 20 PFAS. Interestingly, concerning this total limit, current levels of TFA alone would cause exceedance at the µg/l level.

Our monitoring data revealed that the presence of PFAS in SW is a threat to environmental quality, and to DW sources. Structured mitigation strategies, including the Dutch government's proposal for a complete ban on the production and use of PFAS in Europe this year, are necessary to decrease PFAS emissions into the environment. Producing safe DW from PFAS-contaminated SW is exceedingly challenging, where removing already-present PFAS is a significant burden.

#### **6.12.P-Tu535 Alternatives to the use of per- and polyfluoroalkyl substances (PFAS) in the electrodes and electrolytes of lithium-ion batteries (LIBs)**

*Eleni Savvidou<sup>1</sup>, Amanda Rensmo<sup>2</sup>, Jonathan P. Benskin<sup>3</sup>, Steffen Schellenberger<sup>4</sup>, Xianfeng Hu<sup>5</sup>, Marcel Weil<sup>6</sup> and Ian Cousins<sup>7</sup>, (1)Stockholm University, Sweden, (2)The Environment and Sustainable Chemistry, Uppsala University, Sweden, (3)Department of Environmental Science (ACES), Stockholm University, Sweden, (4)RISE Research Institute of Sweden, Sweden, (5)SWERIM AB, Sweden, (6)Helmholtz Institute Ulm, (7)Department of Environmental Science, Stockholm University, Sweden*

The broad proposed restriction on per- and polyfluoroalkyl substances (PFAS) will inevitably impact the energy sector, that relies on the use of PFAS in various renewable energy technologies. This includes the lithium-ion battery (LIB) sector, where polymeric PFAS are used as well as low molecular weight PFAS, but also other organofluorine compounds. In the PFAS restriction proposal, but also in publicly disclosed documents from industry, it is noted that to date there are no viable alternatives available on the market that could potentially replace PFAS in LIBs. For this study, these claims were examined by reviewing the literature, and interviewing experts from industry and academia in order to assess the current state of science and market regarding (organo)fluorine-free alternatives. The focus was on cathode materials (electrode) and electrolyte components, since these parts of LIBs cover the crucial uses of PFAS. The investigations revealed several companies that developed alternative materials which are PFAS-free/(organo)fluorine-free as well as ongoing research activities towards such materials. One of the biggest current challenges was identified to be the substitution of polyvinylidene fluoride (PVDF), which accounts for the biggest fraction of PFAS-content in LIBs, and supposedly is the material that works best in term of performance. The electrolyte composition does not necessarily rely on the use of organofluorine compounds, but this is dependent on the overall cell chemistry and performance requirements of the final battery application. However, more future investigations and assessments are needed to fully evaluate this and compare the current materials with the available alternatives regarding tradeoffs. Theoretically, it is possible to have a PFAS-free battery, and the PFAS restriction can be an opportunity to strive towards innovation. The overall conclusion is, that it is rather a question to find a balance between optimizing battery performance to combat climate impacts and using sustainable materials to avoid the use of hazardous chemicals, and consequently their adverse environmental impacts.

#### **6.12.P-Tu536 Trade-Offs Between Climate and Perfluoroalkyl Carboxylic Acid Formation: Is it Possible to Minimize Both Global Warming Potential and Persistent Products in F-gases?**

*Cora Young, York University, Canada*

It is well established that certain volatile fluorinated heat transfer fluids, fire suppressants, and solvents (F-gases) can degrade in the atmosphere to form trifluoroacetic acid (TFA). Common F-gases can also degrade to form other perfluoroalkyl carboxylic acids (PFCAs), including perfluoropropionic acid and perfluorobutanoic acid. While certain mechanisms that form TFA as a major product have been well studied, there has been less focus (and a great deal of confusion) on mechanisms that form TFA and other PFCAs as minor products. Recent environmental observations have demonstrated the importance of F-gases to the environmental burden of TFA and other PFCAs. This is likely to continue as F-gases with high global warming potential and relatively low PFCA yield are replaced with F-gases that have low global warming potential and high PFCA yield. I will summarize the state of knowledge regarding atmospheric formation of PFCAs from F-gases. Using the full range of F-gases identified by the Intergovernmental Panel on Climate Change Assessment Report 5, I will identify the structural features that do and do not lead to PFCA formation. Among the same group of molecules, I will also highlight the structural features that underly F-gases with high and low global warming potential. The potential for chemical design that results in no PFCA formation and low global warming potential will be discussed.

#### **6.12.P-Tu537 Assessing the Destruction and Gaseous Carry-over of PFAS during Hydrothermal Carbonization (HTC) of Sewage Sludge**

*Georgia Altiparmaki<sup>1</sup>, Georgia Gatidou<sup>2</sup>, Emma Knight<sup>3</sup>, Ian John Allan<sup>3</sup>, Athanasios Stasinakis<sup>2</sup> and Stergios Vakalis<sup>2</sup>, (1)University of Aegean, Norway, (2)University of the Aegean, Greece, (3)Norwegian Institute for Water Research (NIVA), Norway*

Per- and polyfluoroalkyl substances (PFAS) are characterized by their persistent and stable nature, possessing thermal and chemical stability that renders them suitable for diverse industrial uses. Owing to their extensive application and complex characteristics, PFAS have been identified in soil, water, groundwater, wastewater, and sewage sludge. Certain PFAS, notably PFOA and PFOS, have been established as harmful to both human and environmental health. The application of hydrothermal treatment emerges as a promising technology for PFAS elimination. This method entails subjecting contaminated materials to high temperatures and pressures with water or steam, effectively breaking down PFAS molecules into less harmful compounds like carbon dioxide and water [1]. On the one hand, it has been reported that perfluoroalkyl carboxylic acids (PFCAs) can be reduced via decarboxylation and subsequent defluorination reactions [2]. On the other hand, the nucleophilic substitution and decarboxylation reactions during HTC can lead to the reduction of PFOS [3].

Throughout this process of HTC, sludge undergoes thermochemical transformation in the presence of water under elevated temperature and pressure, yielding hydrochar—a carbon-rich material suitable for energy generation or soil enhancement. It should be stated that this thermochemical pathway produces all the three phases of outputs, i.e. gaseous, liquid and solid that should be studied for their quality. Conventional analysis includes the measurements of PFAS in the solid and liquid phases, but the parameter of PFAS reduction due to carry-over into the gaseous phase has not been studied and is a novelty of this study. In this framework, a unique PFAS-free gas sampling set-up was developed for the condensation of the liquid products, the cleaning of the gaseous products and the collection of PFAS from the gaseous phase on the adsorptive surface of specialized cartridges. The measurements of the HTC products were implemented on a Nexis 2030 GC-BID, while specialized columns for gaseous (RESTEK ShinCarbon 80/100) and liquid phases (J&W Agilent FFAP, MEGA-10) were utilized. An LC - MS/MS was used for the analysis of PFAS on all three phases. Therefore, this study will present the preliminary results of the HTC product analysis and the fate of PFAS on sludges that undergo HTC.

## Track 7. Moving Beyond – Cross Cutting Themes, Emerging and Transdisciplinary Topics

### 7.01 Climate Change and Chemical Contamination: From Combined Effect Studies to Environmental Risk Modelling

#### 7.01.T-01 The Interactive Effects of Temperature and Chemicals at Different Levels of Biological Organization of Aquatic Ecosystems

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This presentation aims to present the interactive effects of temperature and imidacloprid residues at different levels of biological organization. The research has been carried out under the ECORISK2050 project ([www.ecorisk2050.eu](http://www.ecorisk2050.eu)). The ECORISK2050 project applied a holistic approach to address the numerous challenges arising from chemical risk assessment under global change. It was launched with support from the European Union Horizon 2020 program to support the European Union's goal of a non-toxic environment by 2050. Experiments were conducted to study the impact of temperature on the toxicokinetics and toxicodynamics of imidacloprid on individuals of the aquatic arthropod *Gammarus pulex*. These experiments were utilized to parameterize a toxicokinetic-toxicodynamic (TKTD) model which encompasses temperature-dependent parameters. Our findings indicate that toxicokinetic and toxicodynamic parameters scale differently with temperature, calling for further study to unravel the underlying mechanisms. An individual-based model (IBM) was used to evaluate the impact of temperature on the effects of imidacloprid at the population level. This highlights the significance of integrating temperature-sensitive parameterization into population models to conduct reliable risk assessments, considering the projected climate conditions characterized by increased variability. Mesocosm experiments were performed to evaluate the interactive effects at the population, community, and ecosystem levels. The results revealed interactive effects of temperature and imidacloprid, which induced temperature-enhanced and time-accumulative toxic effects, with further implications for the recovery dynamics of different communities and populations, as well as the functioning of freshwater ecosystems. Although we can understand how temperature affects chemical toxicity at the individual level, we have difficulties to understand the effects at the community and ecosystem level. To gain more understanding, we can link the suborganismal level with individual-level approaches like TKTD, population level models such as TKTD based IBMs, and population level approaches with community and ecosystem level models like food web models. Overall, our findings signalize that the health of freshwater ecosystems and other interconnected ecosystems in the future will rely on local and global efforts to mitigate the impacts of these multiple stressors.

#### 7.01.T-02 Combined effects of heat waves and pesticide pollution in aquatic ecosystems: does the timing of stressor matter?

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Most investigations assessing the combined effects of chemical and non-chemical stressors on aquatic ecosystems have been based on simultaneous stressor applications. However, asynchronous exposure scenarios may be more common in nature, particularly for pulsed stressors such as heat waves and pesticide concentration peaks. In this study, we investigated the single and combined effects of the insecticide chlorpyrifos (CPF) and a heatwave (HW) on a zooplankton community representative of a Mediterranean coastal wetland under synchronous (CPF+HW) and asynchronous (HW->CPF and CPF->HW) exposure scenarios using microcosms. The CPF concentration used in the experiments was 0.8 µg/L (single pulse), and the HW was simulated by a temperature increase of 8°C above the control temperature (20°C) for 7 days. The interaction between stressors in synchrony resulted in negative synergistic effects at the population level (*Daphnia magna*) and additive at the community level. In addition, it determined an increase in tolerant species (*Moina* sp.). The abundance decline caused by the asynchronous exposure scenarios at the population level were similar to that observed by the synchronous one; however, the timing of stressor resulted in different responses in the long term. In the HW->CPF treatment, the *D. magna* population recovered faster than in the CPF+HW treatment, probably due to survival selection and cross-tolerance mechanisms. In the CPF->HW treatment, the effects lasted longer, and the population did not recover within the experimental period, most likely due the high energetic costs of detoxification and damage repair, which affected survival and reproduction. Indirect effects in competing species differed among the tested asynchronous scenarios, which were translated into different long-term structural effects at the community level. Our study highlights the relevance of considering the order of stressors to predict long-term effects both at the population and community levels.

#### 7.01.T-03 Future Risks for Migrating Salmonids Exposed to Chemicals from Road Runoff

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Climate change is projected to increase the intensity and frequency of extreme weather events. Prolonged periods of drought will result in more buildup of harmful chemicals on impervious road surfaces. During first-flush events, this chemical buildup can runoff to surrounding streams, leading to higher peak exposures for species in receiving waters. Explicitly linked to runoff from roads is 6PPD-q (6-para-phenylenediamine quinone), an ozonation transformation product of the common tire antioxidant 6PPD. 6PPD-q is lethal to various salmonid species, whose temporal and spatial migration patterns coincide with early autumn first-flush events along the northwestern American Pacific coast. Massive pre-spawn die-offs of coho salmon are well-

characterized and associated with the extent of urbanization and motor vehicle traffic density near spawning habitats. Climate change will increase the frequency, magnitude, and geographical extent of pre-spawn die-off events. To support current and future environmental risk assessment of road runoff-associated chemicals such as 6PPD-q, a spatiotemporal integration of dry spell duration, road intensity and geospatial species occurrence is necessary. We show here that such an integration of local climatic, infrastructural, and ecological information allows for a meaningful first-tier risk mapping for four particularly sensitive salmonid species (rainbow trout, brook trout, white-spotted char and Chinook salmon). Risk maps based on historical species occurrence data (1950-2023) and current climate conditions were in line with georeferenced data on historical pre-spawning mortality in North America. This agreement puts confidence in the risk maps we are currently creating for future climatic conditions. For these risk maps, projections of future dry spell duration are generated using seven global climate models (IPSL, Nor-ESM, HadGEM, GFDL-ESM2M, CSIRO, bcc-csm, ACCESS) from the CMIP5 ensemble for RCP4.5 and RCP8.5. Future road intensity will be projected using the GRIP database (Global Roads Inventory Project). We expect our approach and resulting maps to provide insights in future areas and species of concern, i.e., where drought intensity and timing of first-flush events will coincide with salmonids migrating through increasingly urbanized areas.

#### **7.01.T-04 Adaptation of Metals Risk Assessments to Consider Climate Change**

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Climate change is a complex topic which will have major impacts on the physical and chemical constituents in the environment and may alter the framework upon which environmental risk assessments are conducted. The risk assessment paradigm for metals is unique in accounting for specificities such as essentiality, speciation, partitioning, and bioavailability. The exposure scenarios are further complicated as the push for green technologies in Europe and around the world is inextricably correlated with an increased demand for metals. Events such as temperature rise, ocean acidification and freshwater salinization are just three examples of occurrences linked to a changing climate that have the ability to modify metal toxicity. If the effects of the changing environment are not considered in risk or impact assessments, increased toxicity observations could be attributed solely to metal contamination. This may lead to challenges in compliance assessment, derivation of environmental quality standards, and hazard classifications on a global scale. A general framework for applying environmental risk assessments under different climate scenarios is needed in order to employ best chemical management practices.

Five experts were charged with reviewing the available metals environmental risk assessment guidance materials to identify areas which require alterations to ensure environmental risk assessments remain applicable and accurate as we navigate the changing climate. The exposure scenarios of metals are expected to be altered by changes in both emissions patterns and chemical fate and transport. Characterization of environmental hazards are expected to be impacted through both Climate-Induced Toxicant Sensitivities (CITS) as well as Toxicant Induced Climate Susceptibilities (TICS). In response to these issues, bioavailability models will also require some degree of adaptation to ensure they maintain the capacity to generate relevant predictions for bioavailable metal concentrations and organism toxicity. Several areas have been identified as requiring immediate focus, including a better understanding of temperature impacts and parameterization of organic matter binding properties, and expanded pH boundaries to better account for acidification.

Informed by these conclusions, the authors aim to generate a peer-reviewed scientific article highlighting the necessary changes to the Metals Environmental Risk assessment paradigm and targeted for completion in mid-2024.

#### **7.01.P Climate Change and Chemical Contamination: From Combined Effect Studies to Environmental Risk Modelling**

##### **7.01.P-Th569 Combined Pesticide and Temperature Stress Marginally Affect Leaf Decomposition in an Outdoor Stream Mesocosm**

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The decomposition of allochthonous organic matter is the base of the so-called brown food web, which dominates in headwater streams. Stressors such as pesticides as well as temperature increase are known to affect organic matter decomposition by impacting associated organisms such as aquatic fungi or macroinvertebrate shredders. To be as close to natural systems as possible, we conducted an outdoor stream mesocosm study with repeated pesticide exposure and two temperature levels to investigate effects on microbial and total (microbially and macroinvertebrate-mediated) decomposition at two time points. We hypothesised that microbial and total decomposition will be reduced with increasing pesticide exposure, while the temperature

increase at high exposure levels intensified the reduction but mitigated the effects at low exposure levels. We repeatedly spiked three levels (+ a control) of a realistic mixture containing 13 pesticides were repeatedly spiked to heated (temperature increase ~2.5 °C) and non-heated mesocosms. Per mesocosm, we deployed three fine and coarse mesh bags each filled with black alder leaves (*Alnus glutinosa*, (L.) Gaertn.) for two weeks allowing for microbial and total (microbial and macroinvertebrate) decomposition, respectively, at two time points. We investigated effects on organic matter decomposition, fungal biomass, exo-enzyme activity as well as fungal communities. In contrast to our expectations and previous findings, the temperature normalised microbial organic matter decomposition at the first time point did not differ between different pesticide exposures irrespective of the temperature treatment. The total decomposition also showed no response to pesticide exposure at temperature control levels, which is in contrast to previous findings under controlled conditions and was previously detected in few cases. With temperature increase, however, the total decomposition at intermediate pesticide concentrations was increased in comparison to the controls, while the decomposition in the highest pesticide concentration was slightly reduced. This suggests that macroinvertebrate shredders compensated combined intermediate pesticide stress and temperature increase with higher feeding rates. During the conference, we will present and discuss data of the second time point as well as on responses of the fungal community, the fungal biomass as well as the exo-enzyme activity.

#### **7.01.P-Th570 Robust adaptation to climate change in environmental risk assessments**

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Climate change poses diverse threats to ecosystems, affecting habitat, promoting invasive species, and increasing chemical exposure, disrupting native species' ecological processes. However, anticipating the magnitude of change, and thus the environmental impacts remains highly uncertain. Ideally, risk assessments that seek to adapt environments to the impacts of climate change would do so despite the uncertainty around the degree of climate change. Such adaptation would be considered robust.

One approach to assess climate change impacts and the effectiveness and robustness of adaptation is to use so-called “bottom-up” methods. These attempt to first understand system sensitivity to environmental change. Then, sensitivity of impacts is assessed in the context of information from climate models. These methods have benefits over traditional approaches in that they can show insights into non-linear system behaviour, such as thresholds in future conditions that lead to rapid performance degradation; and by assessing system performance with and without intervention, they can show the range of climate conditions in which adaptation is effective or not.

In a recent study on the Goulburn River basin in Australia, a bottom-up assessment indicated potential rapid decline in ecosystem health without adaptation under a more than 10% decrease in mean precipitation, exacerbated by increased mean temperature. This was well within the range of global climate model projections within the next few decades. One potential adaptation was to restore high flows that provide ecological benefits. Improving high flows increased the resilience of many ecological endpoints beyond their baseline sensitivity to climate change. After intervention, some ecological endpoints could now tolerate up to ~15% reductions in mean precipitation, although there were risks for some endpoints in wetter climates. There was also a degree of plausible climate change (generally >15% reductions in mean precipitation) beyond which the adaptation option did not offer environmental benefits.

Bottom-up methods can help practitioners learn more about their system by yielding insight into performance, sensitivity, and intervention robustness to uncertainty in the range of change of system stressors. Whilst not without their shortcomings, they are a useful tool in risk management approaches in a non-stationary environment.

#### **7.01.P-Th571 Integrating Climate Model Projections into Environmental Risk Assessment: a Probabilistic Modeling Approach**

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SETAC convened a Pellston workshop in 2022 to examine how information on climate change could be better incorporated into the ecological risk assessment (ERA) process for chemicals as well as other environmental stressors. A major impetus for this workshop was that climate change can affect components of ecological risks in multiple direct and indirect ways, including the use patterns and environmental exposure pathways of chemical stressors such as pesticides, the toxicity of chemicals in receiving environments, and the vulnerability of species of concern related to habitat quality and use. This presentation explores a novel modeling approach for integrating climate model projections into the assessment of near- and long-term ecological risks, developed in collaboration with climate scientists. State-of-the-art global climate modeling and downscaling techniques may enable climate projections at scales appropriate for ERA. It is, however, also important to realize the limitations of individual global climate models, and make use of climate model ensembles represented by statistical properties.

Our probabilistic modeling approach combines the projected climatic variables as well as the associated uncertainties from climate model ensembles in conjunction with ERA pathways. We draw upon three examples of ERA that utilized Bayesian networks for this purpose, and represented methodological advancement for better prediction of future risks to ecosystems. We envision that the modeling approach developed from this international collaboration will contribute towards better assessment and managing of risks from chemical stressors in a changing climate, taking into consideration society's multifaceted response to these changes.

The views expressed in this abstract are those of the authors and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.

#### **7.01.P-Th572 Environmental Behavior of a Novel Anti-Corrosion Nanomaterial in a Global Change Scenario**

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Corrosion is the main cause of materials' degradation immersed in seawater. This process is minimized by applying protective polymeric coatings containing efficient anti-corrosion additives. However, such additives are often toxic to the aquatic biota, requiring their replacement by eco-friendly alternatives with good anti-corrosion efficacy. The Zn-Al layered double hydroxides (LDH) were used to immobilize benzotriazole (BTA), a well-known corrosion inhibitor, which can reduce its early leaching and extend the corrosion protection of metallic immersed substrates over time. Since LDH-BTA is a novel anti-corrosion nanomaterial, the present study aims to assess the release of BTA in different conditions, reproducing temperate and tropical environment conditions, in a global change scenario. Three different concentrations of LDH-BTA (1.23, 11.11 and 100 mg BTA/L) were placed at two different temperatures (20°C and 30°C), with different pHs (actual and with a decrease of 0.4 in the pH as predicted by SSP3 – 7.0 IPCC 2021) and in the presence or absence of dissolved organic matter (1 g/L of dissolved humic acid of Sigma-Aldrich) to determine the release profiles of BTA to the overlying water, over time. An aliquot was taken daily for five days and read in the spectrophotometer (275 nm) to estimate the concentration of BTA released into the water. High release of benzotriazole was detected in treatments containing dissolved organic matter at both temperatures, followed by the acidified warm water treatment and the dispersions at the higher temperature. The physical-chemical conditions of the environment can increase the release of the compound from the nanoclay. The present findings raise a very important outcome facing the global changes and the IPCC predictions, showing that extreme scenarios can increase the release of corrosion inhibitors from the nanomaterials with a possible predictable ecotoxicological effects in the marine biota.

#### **7.01.P-Th573 Assessing the dynamics of chemical pollution within urban environments: Toxicological implications for aquatic ecosystems in a changing climate**

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Anthropogenic activities, including chemical pollution and climate change, continuously pressure aquatic ecosystems. Combined, these multiple stressors progressively threaten the resource water as well as freshwater ecosystems, leading to a loss of biodiversity and ecosystem function. However, traditional environmental risk assessment still focuses on individual stressors, ignoring their complex interactions and combined impact. Specifically, the impact of extreme weather events and altered water parameters on interactions between environmental matrices and chemical contaminants in aquatic environments is frequently neglected. Chemical contaminants, typically consisting of complex and unknown mixtures of pollutants, may undergo remobilization or introduction into freshwater environments due to increased flooding and stormwater run-off, altering their toxicological properties. The limited knowledge in our understanding of chemical pollution dynamics in urban aquatic ecosystems in the context of climate change serves as the starting point for this project. As part of the interdisciplinary WATCH project, the present investigations will focus on resulting toxicological implications, seeking molecular and mechanistic insights. The study design will follow a tiered approach. Hence, effect-directed analysis (EDA) with a combination of existing state-of-the-art bioreporter assays and chemical tools will serve as the basis to identify and characterize key toxicants in various environmental samples such as water, sediment, soil, and suspended sediment from selected catchment areas. The development of novel EDA approaches incorporating zebrafish embryos and bioassay-guided fractionation, will be employed to explore mechanism-specific toxicological effects and identify molecular biomarkers, via additional analysis of gene expression, developmental toxicity, and behavioral alterations. Following the tiered approach, in vivo studies using the zebrafish model will offer the opportunity to manipulate water parameters and exposure schemes, effectively simulating climate change effects. The utilization of adult zebrafish in a microcosm study encompasses multiple hierarchical levels of biological organization, including mechanism-specific toxicity, gene expression, reproduction, fertility, and behavioral measurements. Overall, we aim to provide a comprehensive understanding of the complex interactions and combined impacts of multiple stressors on aquatic ecosystems.

#### **7.01.P-Th574 Impact of Global Warming on OECD Water-Sediment Test Systems**

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Water-sediment toxicity tests with *Myriophyllum spicatum* (OECD test guideline 239), *Chironomus riparius* (OECD 218/219) and *Lumbriculus variegatus* (OECD 225) are conducted at  $20 \pm 2$  °C.

However, in the light of global warming it needs to be questioned whether increased water temperatures influence growth and development of test organisms in a water-sediment system and could therefore lead to a shift of toxicity endpoints.

To evaluate possible effects of higher temperatures, toxicity tests with *Myriophyllum spicatum* (OECD 239), *Chironomus riparius* (OECD 219) and *Lumbriculus variegatus* (OECD 225) were conducted at  $20 \pm 2$  °C and  $24 \pm 2$  °C. For each test system, both control growth/development and toxicity endpoints of the tests run at two different temperatures were compared to evaluate possible differences and thereby impacts of a higher temperature on the results of toxicity tests.

#### **7.01.P-Th575 Evaluating Combined Effects of Temperature and Chemical Exposure in *Daphnia magna***

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Assessing the risks and impacts of contaminants in aquatic environments requires a comprehensive understanding of combined effects involving environmental factors and chemical exposure. Temperature modulates virtually all physiological functions and often affects contaminant-organism interactions. Moreover, biological response to chemical exposure depends on the exposure duration. Hence, test temperature may alter both the exposure duration on the physiological time scale and chemical toxicity by affecting toxicokinetics. Here, we used a novel combination of the degree-days (DD, a proxy for physiological age) approach and the chemical activity concept to evaluate interactions between chemical exposure and thermal stress in the model organism *Daphnia magna*. In an acute toxicity test, the chemical activity range causing narcosis (0.01 – 0.1) was generated using a mixture of polycyclic aromatic hydrocarbons (acenaphthene, phenanthrene, fluoranthene, and fluorene) at two exposure temperatures (20 °C and 25 °C). We hypothesized that: (1) lower median lethal activity ( $La_{50}$ ) occurs at the higher temperature if the exposure time is defined in calendar time (3 days) because of the temperature effects on the metabolic rate and time-to-toxicity, and (2) there is no temperature effect when the exposure time is defined in degree-days (corresponding to 60 DD) representing the physiological time at different temperatures. Our results confirmed a significantly lower  $La_{50}$  at 25 °C than at 20 °C (0.035 vs 0.068) after 3 d, whereas no significant difference was found when time was defined in DD (0.061 vs 0.068). Thus, the expected rise in toxicity at higher temperatures during the 3-day exposure period was confirmed. Moreover, the temperature controlled the toxicity rate (time to death) with no significant temperature  $\times$  dose interaction, as indicated by similar  $La_{50}$  values for physiologically equal exposure durations. Therefore, when testing combined effects of temperature and toxic exposure, the exposure time should be adjusted to the physiological age of the test animals if we are to detect non-additivity of these effects for risk assessment, especially in the context of climate change and chemical pollution.

#### **7.01.P-Th576 Impact of climate change on effects in *Daphnia* acute studies**

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Acute toxicity tests with *Daphnia magna* (OECD test guideline 202) are currently conducted within the temperature range of 18 to 22 °C. This is considered the optimum temperature range for this organism.

However, in the light of global warming it needs to be questioned whether increased water temperatures outside its optimum range will affect the ability of this organism to adapt and continue to be used as the surrogate for tier 1 acute toxicity tests for aquatic invertebrates. Or could increased temperatures outside its optimum range create a scenario that could influence the effects of chemicals towards daphnids and could therefore, lead to a shift of toxicity endpoints?

To evaluate possible impacts of higher temperatures, acute toxicity tests with *Daphnia magna* (OECD 202) were conducted at 20, 23, and 25 °C. Tests included daphnids that were acclimated for a period of time to the new test solution temperatures and other tests where daphnids were transferred directly from optimum temperature solutions to test solutions at the higher temperatures. At each temperature boric acid and potassium dichromate were tested. Both, control immobilization and toxicity endpoints of the tests runs at the three different temperatures were compared to evaluate possible differences and thereby impacts of higher temperatures on results of toxicity tests with *Daphnia magna*.

#### **7.01.P-Th577 The Effect of Combined Environmental Stressors on *Daphnia magna* and *Brachionus calyciflorus*.**

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Multiple stressors pose a complex challenge to the environmental health of aquatic ecosystems. Due to climate change and increased anthropogenic influences, ecosystems are exposed to a variety of chemical and non-chemical stressors. However, most ecotoxicological studies focus on single stressors, neglecting the synergistic, additive, or antagonistic effects of multiple stressors. This makes it difficult to identify the main drivers of changes in aquatic ecosystems. Water quality parameters such as temperature, pH, and salinity are critical for the survival of an organism and can act as stressors when not present in optimal ranges. The upper and lower tolerance limits for these stressors have been studied individually for the sake of drafting policies

and ecotoxicological tests with respect to various model organisms. However, in an ecosystem, these prevailing water quality parameters may be at sub-lethal or lethal levels and may have a synergistic, additive or antagonistic effect on each organism's sensitivity or tolerance towards them. This study delves into the combined effect of these three stressors on two model organisms used in freshwater ecotoxicological tests: *Daphnia magna* and *Brachionus calyciflorus*. The organisms were exposed to a pH range of 5, 6, 7, 8, 9 in combination with salinities of 0.5, 1, 2.5 and 5 PSU at 4 different temperatures- 15°C, 20°C, 25°C and 30°C for an acute test of 48hrs. The mortality was recorded every 12hrs to identify temporal trends. The ongoing study aims to elucidate how different combinations of non-chemical stressors affect organisms over time during acute exposures and is anticipated to provide valuable insights into ecologically relevant, multi-stressor conditions.

#### **7.01.P-Th578 How Pyrene and Salinity Affect the Thermal Tolerance Thresholds of Copepods**

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The ocean is warming at a rate unprecedented over the past ten thousand years, and extreme warming events are both increasing in frequency and intensity. This could surpass the thermal tolerance threshold of marine species, a key tipping point that, when exceeded, can lead to abrupt and irreversible consequences. At the same time, the intensification of extreme weather events and human activities increase the exposure of marine organisms to decreasing salinities and emerging contaminants such as polycyclic aromatic hydrocarbons (PAHs). As organisms are simultaneously exposed to multiple stressors in their natural environment, it is therefore crucial to identify the effects these stressors have when combined to project their future in a changing world. The present study aims to investigate how multiple stressors (salinity and the PAH pyrene) affect the thermal thresholds of marine zooplankton. Copepods are key planktonic organisms and were selected as the study species due to their essential role in energy transfer in the marine food web. The first experiment aimed to determine how oil contamination alters the thermal tolerance threshold of *Chiridius* copepods. As a proxy for oil, the copepods were exposed to the PAH pyrene, at 5 different concentrations and at 7 different temperatures for a duration of 14 days. The second experiment aimed to determine how decreasing salinities alter the thermal threshold of *Calanus* copepods. The copepods were exposed to 5 different salinities at 5 different temperatures for a duration of 13 days. Copepods for both experiments were collected in the Oslofjord, Norway, and survival was checked every day during the experiments. For both experiments, increasing pyrene concentration and decreasing salinity were expected to reduce the copepods tolerance to warming. Findings from the study will provide important insights to the effects of multiple stressors and how it affects physiological tipping points.

**Key words:** Copepods, Multiple stressors, Tipping point, Reaction norm, *Calanus*, *Chiridius*, Marine heatwaves, Pyrene, Salinities

#### **7.01.P-Th579 Influence of temperature and nutrient availability on the toxicity of the fungicide trifloxystrobin to the aquatic hyphomycete *Articulospora tetracladia***

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Fungicides represent 40% of pesticide sales in the European Union, therefore the possibility of these products reaching aquatic ecosystems following their application on agricultural fields is high. At the same time, studies that assess the responses of non-target aquatic fungi to fungicide exposure are scarce. In this context, we evaluated the toxicity of the fungicide trifloxystrobin to the aquatic hyphomycete *Articulospora tetracladia* at three temperatures (T1=12 °C, T2=16 °C and T3=20 °C) and nutrient levels (F1=0% w/v, F2=0.5% w/v and F3=1.5% w/v) in agar medium. For this, we used five trifloxystrobin concentrations: 0, 1, 5, 25, 125 and 625 µg/L, each being replicated five times over 21 days. Fungal radial growth was measured using the software ImageJ. Effective concentrations (ECx) reducing growth by 10 and 50% at study termination were calculated ("drc" package) in parallel to traditional hypothesis testing (two-way ANOVA) using R version 4.2.2. The ECx values varied between temperatures and nutrient levels. At T1, T2 and T3 the EC10 and EC50 values were F1=128 and 877; F2=16 and 597; F3=17 and 332 µg/L; F1=97 and 834; F2=2 and 377; F3=32 and 589 µg/L; F1=12 and 553; F2=10 and 460; F3=15 and 223 µg/L, respectively. The ECx values showed that growth tended to be more affected at higher nutrient levels. Fungicide toxicity is also positively related to temperature, meaning that growth inhibition increased at higher temperatures. Regardless of the fungicide effects, growth increased with temperature and nutrient availability. Comparisons between the different nutrient levels at the same temperature and fungicide concentration showed no significant differences in fungal growth at 12 °C and 16 °C ( $p > 0.05$ ). At 20 °C, in contrast, all comparisons were significant ( $p < 0.0001$ ). Between temperatures, all comparisons between 12 and 16 °C and 12 and 20 °C were significant ( $p < 0.0001$ ). Between 16 and 20 °C the comparisons are also significant, with the exception between growth at F1 and F2. In summary, the impact of trifloxystrobin is temperature and nutrient dependent. The interaction of high temperature and nutrient levels can influence absorption, distribution and detoxification of the fungicide potentially explaining the results. This may represent a greater risk for *A. tetracladia* in regions with higher temperatures provided sufficient levels of nutrients are available, both of which can be assumed in (sub)tropical agricultural landscapes.

#### **7.01.P-Th580 Mixture Toxicity of Two Pesticide Formulations on Aquatic Mesocosm Communities**

**Jonas Nelles, Juliane Filser, Leonard Otterbein and Alica Rother, University of Bremen, Germany**

Pollution of surface water bodies with pesticide mixtures in agricultural landscapes is problematic because tank mixtures

applied to fields often contain several pesticide formulations. Formulations are mixtures of active ingredients and adjuvants in undisclosed composition that alter bioavailability and therefore toxic effects of the product. Although pesticide formulations are more realistic, toxicity data is lacking, because most exposure experiments are conducted with active ingredients instead of formulations and often assess acute toxicity in laboratory conditions. The need for more realistic exposure scenarios is evident.

We set up a long-term experiment with aquatic mesocosms simulating a freshwater ecosystem. The mesocosms were incubated with sediment from a nearby lake and cladocerans from laboratory cultures. Two pesticide product formulations in common use were chosen to simulate a worst-case exposure scenario with renewed application throughout the year: a herbicide containing glyphosate as an active ingredient and the fungicide Funguran progress (FP), relying on the fungicidal activity of  $\text{Cu}(\text{OH})_2$  in nanoparticle form. Both products were applied individually and in mixture.

Preliminary results indicate a shift in the macrozoobenthos community over time in all treatments. Cladocerans were the organism group with the highest abundance at the start of the experiment, while the number of copepods and ostracods increased with ongoing succession of the mesocosms. Over the course of the experiment, more and more organism groups were detected in samples of the water column, including gastropods, turbellarians and larvae of nematocera, ephemeropterans and odonates. Principal component analysis showed stable trends of copepod and ostracod abundance inversely correlating with glyphosate treatment, but copepods seem to correlate positively with FP. On the other hand, FP seemed to have a negative impact on abundance, at first on ephemeropteran and nematoceran larvae, and later on gastropods, turbellarians and odonate larvae. Algal blooms also impacted the community, as in spring samplings, cladocerans and nematocera correlated with occurrence of algae in the mesocosms, likely because they act as a food source or microhabitat. However, variation in the same treatments was high due to differences in physico-chemical parameters like light and nutrient influx. Results from samplings in spring of 2024 will be included.

#### **7.01.P-Th581 Effects of the Herbicide Terbutylazine on Freshwater Communities Under Different Global Climate Change Warming Scenarios**

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Due to Global Climate Change (GCC), we expect rising temperatures and more often reoccurring heatwaves in aquatic ecosystems every year. Previous studies have found that warming may influence the toxicity of chemicals (*e.g.*, pesticides) in the environment by complex mechanisms not fully yet understood. However, in standard ecotoxicological testing warming conditions are not usually considered, and when considered, only as a fixed constant temperature, not representing natural fluctuating scenarios. Pesticides are common chemicals that pollute aquatic ecosystems through various pathways such as spray-drift, agricultural runoff, and chemical spills. Nonetheless, there is a lack of studies evaluating the possible combined effects of pesticides and warming. Terbutylazine (TBA) is a photosynthesis inhibiting herbicide that is globally used as alternative to the more toxic and persistent atrazine. The aim of this study was to assess the single and combined effects of the herbicide TBA and GCC warming scenarios (fluctuating elevated temperatures and extreme heatwave events) on freshwater communities under a realistic set up (outdoor mesocosms). The experiment consisted of a full factorial design with 9 treatments, combining 3 temperature scenarios (ambient, elevated temperature (+4°C) and recurring heatwaves (+8°C for 1-week intervals)) with 3 TBA concentrations (0, 25 and 250 µg/l) over a period of 8-weeks during late summer 2023. Direct and indirect effects on different trophic levels including plankton, macroinvertebrates and macrophytes communities were evaluated. The main endpoints assessed included: biweekly samples of phytoplankton, zooplankton and periphyton; macrophytes coverage and dry weight; and *in-situ* identifications of macroinvertebrates community. Preliminary results show clear effects from the highest concentration of TBA on the dissolved oxygen levels, total algae concentration, and macrophytes' growth. Combined effects at low TBA concentrations seem to be antagonistic but further analyses will be conducted. We hope that the results from this study allows us a better understanding of the mechanistic pathways of these stressors' interactions for a more accurate risk assessment in the future.

#### **7.01.P-Th582 Ecotoxicity Assessment of the Natural Dye Alizarin Using Aquatic Organisms**

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Alizarin is a natural dye from the anthraquinone class. It has been used as an acid-base indicator and as a dye in several industrial processes including textiles. Therefore, they are potential environmental contaminants. Alizarin is mutagenic to bacteria in the Salmonella-microsome test with the addition of S9 metabolic activation and was toxic to the *Lemna gibba*, phytoplankton and zebrafish. Furthermore, in the aquatic environment, anthraquinones can undergo a photosensitization reaction, releasing reactive oxygen species, which can cause photoinduced toxicity. The aim of this work was to complement aquatic toxicity data using the microalgae *Raphidocellis subcapitata*, the freshwater microcrustacean *Daphnia similis*, the marine amphipod *Parhyale hawaiiensis* and embryos of the freshwater fish *Danio rerio*. Also, the effect of light on its acute toxicity to *D. similis* was studied. Alizarin was dissolved in DMSO to its solubility of limit (1.93 g/L) and tested at the maximum concentration of 0.01% DMSO in the media as recommended by OECD in concentration response experiments. A 72-hour exposure chronic toxicity test was performed with *R. subcapitata*. Acute toxicity tests with *D. similis* were carried out for 48h with photoperiod of 16:8h, constant light and dark. Acute tests with *P. hawaiiensis* and *D. rerio* were performed for 96h and 168h, respectively, with photoperiod of 12:12h. Data was analyzed applying generalized logistic models. Alizarin did not inhibit the growth of *R. subcapitata* or cause lethal effect for *P. hawaiiensis* ( $\text{IC}_{50}$  or  $\text{LC}_{50} > 193 \mu\text{g/L}$ ). For *D. similis*,  $\text{EC}_{50}$  of

alizarin was  $90.3 \pm 0.3 \mu\text{g/L}$ . Constant light did not alter the toxicity ( $\text{EC}_{50} 105 \pm 55.8 \mu\text{g/L}$ ) but in the dark, the toxicity increased ( $\text{EC}_{50}$  of  $68.6 \pm 1.2 \mu\text{g/L}$ ). For *D. rerio*  $\text{LC}_{50}$  was  $39.6 \pm 7.2 \mu\text{g/L}$ . Alizarin can be classified as category 1 for aquatic toxicity in the Global Harmonization System (GHS). We concluded that *D. rerio* was the most sensitive organism and light did not increase the toxicity of this anthraquinone, at least for *D. similis*. Data were considered relevant and reliable after Criteria for Reporting and Evaluating ecotoxicity Data (CRED) evaluation and can be used in the future for Predicted No-Effect Concentration (PNEC) derivation.

#### **7.01.P-Th583 Global change effect: when the inter-individual variability provides information about metabolic response in a sentinel species facing extreme events and multi-metallic contamination**

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Global change issues are new challenges in ecotoxicology. The frequency and the magnitude of heat waves still increasing, and a significant relation was established between the occurrence of extreme events and global changes according to the last GIEC rapport. The basal metabolic activity of ectotherm organisms could be strongly affected by quick temperature variations, limiting thus their capacity to face additional stresses. This study aimed to assess the combined effects of a multi-metallic stress and recurrent heat waves in a freshwater bivalve, *Dreissena polymorpha*. Mussels were exposed to an environmental concentration of a nickel-chromium mix solution (20 and  $5 \mu\text{g.L}^{-1}$ , respectively) for 35 days. A weekly gradual increase of the temperature ( $+15^{\circ}\text{C}$ ) was performed in exposed media for 24 hours. Key metabolism functions were targeted along the biological organisation levels through a panel of markers involved in the energy metabolism, defence mechanisms and cellular damage. Different patterns of physiological responses were highlighted between males and females. Surprisingly, the main factor structuring the biological responses differed according to the biological organization level considered. Regarding the overall dataset, a significant effect of the accumulation of stress was observed through an increasing inter-individual variability. This study pointed out the need to consider (1) the gender of organisms, (2) the response at different biological organisation levels, and (3) the inter-individual variability. Hence, global change induces multifactorial stress. To assess its impacts, we need to gather a higher number of markers, encompassing the diversity of biological systems, even if it leads to patterns that are complex to analyse.

#### **7.01.P-Th584 Using scenario predictions of climate, chemical, and physical stressors for probabilistic effect assessment of nearshore coral reefs**

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Near shore reef systems of the Mackay Whitsunday coastal zone are particularly at risk to changes in climate and other anthropogenic stressors. These stressors include chemical, nutrient and sediment exposures that might increase in the future with more frequent and large rainfall events that enable runoff of contaminated waters. We aimed to examine the combined effects of climate change and chemical exposures which is essential for improving environmental risk assessments of vulnerable ecosystems such as the Great Barrier Reef. During the SETAC Pellston workshop on incorporating climate change into environmental risk assessment, we explored the incorporation of changing environmental variables into effect assessment by developing a Bayesian network that quantitatively compares the effects of historic and future climate on inshore hard coral bleaching, mortality, reproduction, and cover. Part of these efforts involved the use of various projection models whose outputs were used to parameterize the developed Bayesian network. We used various prediction models to project environmental variables/ parameters such as precipitation, temperature, streamflow, nutrient, and sedimentation loads to derive prior probabilities. For example, we used an ensemble of 16 downscale models representative of current climate conditions and future climate conditions assuming medium and high carbon emissions for two thirty-year periods centred on 2040 and 2085. The projections used showed an increase in air, sea surface temperature, and bleaching events, and a decrease in nutrient, sediment and pesticide loads for all used future scenarios. The derived future scenarios enabled us to examine the risk multiple stressors pose to inshore reef systems. Under the given assumptions, an increase in coral bleaching and coral mortality under all future climate scenarios was predicted by the Bayesian network. Disclaimer: The views expressed in this abstract/presentation are those of the authors and do not necessarily represent or reflect the views or the policies of the U.S. Environmental Protection Agency.

#### **7.01.P-Th585 Projections of Future Climates, the Problem of Interacting Stressors, Endpoints and Management Uncertainties, and the Estimation of Ecological Risk at Appropriate Management Scales**

*Wayne G. Landis, Western Washington University*

A special series has recently been published highlighting the importance of climate change in conducting ecological risk assessment. In the case studies and the description of methods it became clear that climate change is an important factor in the estimation of risk to a variety of aquatic habitats both alone and in conjunction with other stressors. Climate projections are constructed using a set of models based on first principles and set to predict global conditions. In contrast, risk assessments are performed for specific stressors upon set receptors-endpoints. The physical scale of these assessments can be at extent of a contaminated CERCLA or RCRA site, a watershed or landscape, a specific geographic region or to continental expanse. The species and their range also vary, from very limited spatial and temporal scales, from small critical habitats to joint oceanic and

mountain ranges as in the case of salmonids. A specific challenge is the matching of the climate change projections with the scales appropriate both to the species involved and the management cycle. A major issue with climate projections has been adjusting the scales in time and space to forecast the amount, distribution, fate and transport, exposure and effects of contaminants to populations also exhibiting dynamics affected by historical events, life-history strategies, the distributions of habitat, genetics and human management. The epistemic (knowledge), aleatory (variability) and linguistic (communication) uncertainties in the models need to be understood and integrated. To ensure the use of these risk assessments into an adaptive management program the dynamics of that decision making process, and those uncertainties, need to be considered. Probabilistic methods are necessary to describe and manage the coupling of these factors. A number of probabilistic methods exist but this presentation will focus on Bayesian networks and several case examples-including but not limited to chemical contaminants. A strategy will be presented to integrate the uncertainties into an adaptive management process. The strategy starts with a careful and quantitative delineation of the management goals, the time periods involved and the specific management targets before either the establishment of a climate projection and risk assessment process. Model approaches are suggested, testing of the models described, and the operation of the adaptive management cycle presented.

#### **7.01.P-Th586 Taking account of climate change in FOCUS PEC<sub>sw</sub> calculations**

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For the pesticide risk assessment in the EU, predicted environmental concentrations need to be calculated in order to perform a risk assessment for non-target species. For the aquatic risk assessment, predicted surface water concentrations (PEC<sub>sw</sub>) are calculated using FOCUS models according to current guidance. The weather files used in these models include data from several decades ago. For example, the weather files used in the latest version of FOCUS TOXSWA, used to calculate PEC<sub>sw</sub> values, still date back to the period from 1974-1994. Hence the current aquatic exposure assessment is based on weather data which are about 40 years old and may not be representative for present conditions anymore. In addition, during the last years weather changes have become even more rapid, hence it can be expected that the currently used weather data become even less representative. Overall, one may question the relevance of the exposure assessment if data are not updated. We therefore evaluated the weather data used in FOCUS surface water scenarios and compared them to recent weather data. Furthermore, we also compared PEC<sub>sw</sub> values obtained with the currently used old weather data from 40 years ago with those obtained using recent weather data, using the FOCUS PEC<sub>sw</sub> automation system AutoPEC SW. We conclude that it may be appropriate to update weather data regularly for the risk assessment.

#### **7.02 In Silico Approaches Toward Safer Use and Green Design of Chemicals: Present Achievements and Future Challenges**

##### **7.02.T-01 Comparative Toxicity of Perfluoroalkyl Substances for Safe-by-Design: Quantitative Adverse Outcome Pathway using Bayesian Network Model**

*Jaeseong Jeong, Hyunwoo Kim and Jinhee Choi, School of Environmental Engineering, University of Seoul, Korea, Republic of (South)*

Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals that have been widely used in industrial and consumer products. However, there still exists a knowledge gap in our understanding of the toxicity of the newly developed PFAS. A Bayesian Network (BN) is a model that presents probabilities of the dependencies between any two variables in a graphical form. It is capable of diagnostic, predictive, and inter-causal reasoning. In this study, the toxicity of 12 PFAS [Perfluoroalkyl sulfonic acids (PFSAs) with chain lengths C4-C8 and perfluoroalkyl carboxylic acids (PFCAs) with chain lengths C4-C14] were compared by employing BN model, focusing on variations in carbon chain length and functional groups. First, DNT AOP was developed based on common KEs of AOPs (AOP ID: 12, 13, 17, 48, 54, 260, 281, 442, 475, 483) from AOP Wiki, and KE relationships were adjusted by incorporation of existing knowledge from the literature. And experiments were performed on selected KEs using human neural stem cells. To determine which properties of PFAS affect toxicity, comparisons were made based on the number of carbon chains and functional groups linked to the active events in the BN model. Within the carbonate group, the larger the number of carbon chains, the lower the cell viability. Additionally, the sulfonate group was found to have a greater impact on low cell viability than the carbonate group. The results were not predicted based merely on the effects on cell death but also considered changes in the probability of toxicity pathways through the quantitative AOP concept, making them more reliable and explainable. This approach will promote the utilization of the Safe-by-Design concept for the safe development of PFAS.

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Keywords: ToxCast, Machine Learning, Molecular fingerprints, Toxicity Screening, Toxicity prediction

##### **7.02.T-02 First Do No Harm - A Framework for Intelligent Design of Safer Chemicals**

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In the last 200 years of performing synthetic chemistry, the main goal of chemists was to create chemicals and materials with a

particular function or purpose as a focus. This led to the creation of wonderful products such as effective medicines, plastics, and fertilizers; however, it has also become obvious that these products can be toxic and cause harm to human health and the environment.

Green chemistry introduced safer chemical design as one of the core principles where the focus of synthetic chemistry should be adjusted to avoid present and future hazardous practices. The sensible way of creating less hazardous chemicals lies in applying knowledge gained in both fields of chemistry and toxicology over the decades, but even today, only a small number of chemical departments around the world offer integrated courses on molecular toxicology. Molecular insights into the causes of inherent hazards and ways to avoid them, lessons learned from practices in the pharmaceutical and pesticide industry, and numerous studies conducted to protect the environment offer a knowledge base to outline a conceptual framework for designing safer chemicals and materials. Based on this knowledge, it is possible to determine the relationship between the inherent properties of chemicals and the different types of hazards they can cause.

The basic guidelines on how to design safer chemicals can be represented through a molecular design pyramid, a symbolic representation of the factors that have an impact on different types of hazards that molecules can cause. The factors are arranged in terms of complexity, with the physicochemical properties at the base of the pyramid, the shape and structure of the chemicals higher up the pyramid, and the most intricate factors influencing the mechanism of action at the top. All of these factors can be predicted or simulated in silico, using a wide variety of methods and approaches used in computational chemistry and computational toxicology, which opens a path to the chemicals and materials that will have desired properties and behavior, with inherent hazard minimized or completely eliminated

#### **7.02.T-03 The OECD (Q)SAR Assessment Framework for REACH Dossier Evaluation**

*Andrea Gissi and Doris Hirmann, European Chemicals Agency (ECHA), Finland*

The OECD has published the (Q)SAR Assessment Framework (QAF) in 2023. The QAF is a guidance for the regulatory assessment of QSAR models, predictions, and results based on multiple predictions. The guidance is the result of a two-years project led by the European Chemicals Agency (ECHA) and the Italian Institute of health (ISS). The guidance is complemented by a checklist of assessment elements to consider when evaluating the validity of the (Q)SARs. In general, the framework is flexible enough to be applicable irrespective of the endpoint, modelling technique and regulatory purpose of use of the (Q)SAR. After the OECD publication, authorities are responsible for the application of the framework under the regulations they oversee.

The EU regulation for the safe use of industrial chemicals REACH allows the submission of (Q)SAR results as an adaptation to standard information requirements. ECHA has been assessing (Q)SAR submitted in REACH registration dossiers since 2007, and its experience has been reflected in the QAF. In fact, the framework is aligned with ECHA's current practices for (Q)SAR evaluation. Nonetheless, the publication of the QAF is a good occasion for ECHA to clarify further how (Q)SAR results are assessed under REACH dossier evaluation.

This presentation will discuss how ECHA evaluates the principles for validity and assessment elements listed in the QAF with illustrative examples tailored for environmental endpoints. The examples will cover the evaluation of (Q)SAR results against the principles listed in the QAF: a correct input, the substance falls within the applicability domain of a model, the prediction is reliable, and the outcome is fit for the regulatory purpose.

#### **7.02.T-04 In Silico Gut Metabolism Model for Enhancing New Approach Methods (NAMs) towards Safer Use of Chemicals**

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Chemical risk assessment increasingly relies on animal-free New Approach Methods (NAMs), utilizing in vitro assays and in silico computations. In vitro assays measure chemical concentrations in incubation media rather than in the body, necessitating reliable estimates of internal chemical concentrations from external exposure doses. Pre-absorption intestinal and hepatic biotransformation, also known as the 'first-pass effect,' reduces the entry of orally ingested chemical contaminants into the systemic circulation. While in silico high-throughput physiologically based toxicokinetic (PBTK) models consider hepatic biotransformation, intestinal biotransformation, also known as gut metabolism, is often overlooked, and the relative importance of pre- and post-absorptive biotransformation is unclear. Experimental evidence has shown rapid intestinal biotransformation, e.g., with a half-life at a level of minutes for certain phthalate esters in metabolism by lipases. Neglecting intestinal biotransformation may result in a substantial overestimation of exposure to chemicals in high-throughput chemical screening. In this presentation, using an in silico PBTK model, we evaluate (i) the relative importance of pre- and post-absorptive biotransformation in controlling the presence of chemical contaminants in the human body and (ii) important factors that govern such relative importance. Since in vivo biotransformation data are often inadequate for chemical contaminants, the model also includes an in vitro to in vivo extrapolation module to allow the use of in vitro data. The model shows that the relative importance of the rates of intestinal biotransformation and epithelium permeation greatly impacts intestinal absorption efficiency. In cases of phthalates where biotransformation occurs at a level of minutes, more than 60% of the ingested amount is biotransformed in the gastrointestinal tract before absorption. Overall, our work highlights the importance of intestinal

biotransformation in human exposure and toxicokinetic modeling. This model also enables us to more accurately screen the absorption efficiency of chemicals through dietary and non-dietary ingestion.

## **7.02.P In Silico Approaches Toward Safer Use and Green Design of Chemicals: Present Achievements and Future Challenges**

### **7.02.P-We551 Bridging Gaps, Recognizing Limits: Machine Learning in Chemical Toxicity Characterization**

*Kerstin von Borries<sup>1</sup>, Hanna Holmquist, PhD<sup>2</sup>, Marissa B Kosnik<sup>3</sup>, Katie V Beckwith<sup>4</sup>, Olivier Jolliet<sup>1</sup>, Jonathan Goodman<sup>4</sup> and Peter Fantke<sup>5</sup>, (1)Technical University of Denmark (DTU), Denmark, (2)Swedish Environmental Research Institute (IVL), Sweden, (3)Department of Environmental Toxicology, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Switzerland, (4)Department of Chemistry, University of Cambridge, United Kingdom, (5)Quantitative Sustainability Assessment, Department of Environmental and Resource Engineering, Technical University of Denmark, Denmark*

Global chemicals management is under pressure as the capacity for assessment cannot keep pace with the ever-increasing number and production of chemicals. Hence, there is an urgent need to increase the chemical coverage and reliability of tools that characterize chemical toxicity impacts. An essential roadblock is the lack of chemical property data these tools require to describe fate, exposure, and effects. Machine Learning (ML) is a promising technique frequently applied to improve predictions across scientific fields. However, the systematic adaptation for chemical toxicity characterization is still limited as the potential for developing ML-based approaches that can predict property data for the wide range of marketed chemicals is unknown. This work aimed to prioritize input parameters in toxicity characterization tools for developing ML-based approaches and assess the predictive potential of these approaches to fill input data gaps for diverse chemical structures. We prioritized input parameters to inform ML model development based on two criteria: (1) each parameter's relevance to robustly characterize chemical toxicity based on the uncertainty in characterization results attributable to each parameter, and (2) the potential for ML-based approaches to predict parameter values for a wide range of chemicals based on the availability of chemicals with measured parameter data. Characterization uncertainty attributable to each parameter was evaluated with an uncertainty analysis using USEtox 3.0 beta. Data availability was assessed based on the number of chemicals with measured parameter data available across large data repositories. Based on our analysis, we prioritized 13 out of 38 USEtox input parameters for developing ML-based approaches. In addition, nine parameters showed high relevance for obtaining robust characterization results but had low data availability and were, therefore, flagged as crucial data gaps. For all prioritized parameters, we performed a chemical space analysis that further assessed the potential for ML-based approaches to predict data for diverse chemicals, taking into account the structural diversity of available measured data. The analysis showed that ML-based approaches can potentially predict 8-46% of marketed chemicals based on 1-10% with available measured data. Our results can systematically inform future ML model development efforts to address data gaps in chemical toxicity characterization.

### **7.02.P-We553 Endocrine Disrupting Chemical (EDC) Screening of Additive Chemicals in Plastics Using In vitro ToxCast Data, In Silico Molecular Docking and QSAR Models**

*Kimoon Na, Donghyeon Kim and Jinhee Choi, School of Environmental Engineering, University of Seoul, Korea, Republic of (South)*

Plastic additives are utilized in plastic products to enhance their properties and durability. However, their potential as Endocrine-Disrupting Chemicals (EDCs) remains largely unexplored. This study aims to screen plastic additives for their potential to act as EDCs by analyzing their binding affinity to EDC-related receptors, such as estrogen, androgen, and thyroid hormone receptors. Initially, 173 chemicals used as plastic additives were compiled from the ECHA database. Their bioactivities on EDC related receptors were analyzed in ToxCast bioassays. Among these, 95 chemicals lacked bioactivity data for all EDC-relevant receptors. To address this gap, we utilized in silico molecular docking (MD) simulations and quantitative structure-activity relationship (QSAR) models to predict their potential bioactivity with EDC-related receptors. Binding affinity for docking analysis was conducted using AutoDock Vina software and QSAR models were trained using six machine learning algorithms (gradient boosting tree (GBT), random forest (RF), multi-layer perceptron network (MLP), k-nearest neighborhood (kNN), logistic regression (LR), and naïve Bayes (NB)) and five molecular fingerprints (MACCS, Morgan, Layered, RDKit, and Pattern). As a result, 48 chemicals were predicted to be active in at least one receptor across MD and QSAR models across all target receptors, with 11 of them predicted to be highly active across all receptors. Consequently, 67 chemicals were prioritized using in vitro ToxCast data and in silico modeling. Considering that these chemicals are reported to have high-volume production—exceeding 100 tons per year—further, more comprehensive EDC assessments are needed. Our research highlights both the potential and challenges associated with using in silico MD simulations and QSAR-based predictions as rapid screening tools for industrial chemicals, such as additive chemicals in plastics.

**Keywords:** Plastic Additives, Molecular Docking, Quantitative Structure-Activity Relationship, Endocrine Disrupting Chemicals, ToxCast bioassay

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### 7.02.P-We554 Chemical Space Covered by Applicability Domains of Quantitative Structure-Property Relationships and Semi-empirical Relationships in *in silico* Chemical Assessments

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Thorough and defensible hazard and risk assessments are crucial prerequisites for the safer usage and green design of chemicals. In this context, *in silico* computational models, particularly mechanistic models based on (1) chemical partitioning and reactivity properties and (2) quantitative descriptions of chemical, physical, physiological, and toxicokinetic processes, are instrumental in achieving this objective. Nonetheless, for most chemicals detected in environmental samples or commercialized in the marketplace, there are insufficient experimentally measured chemical property data. In this context, it is necessary to use prediction of chemical property data applying (1) quantitative structure-property relationships (QSPRs), which correlate a chemical property of interest with variables describing chemical structure or molecule-level interactions, such as OPERA, EPI Suite, IFS-QSAR, and QSARINS, and (2) semi-empirical relationships, which correlate a chemical property of interest solely with other properties, without considering chemical structure or molecule-level interactions, such as Karickhoff relationships that predict  $K_{OC}$  from  $K_{OW}$ . Furthermore, it is imperative to evaluate the applicability domain (AD) of QSPRs and semi-empirical relationships to ensure their suitability for regulatory assessment purposes. In this presentation, we systematically reviewed the AD coverage of commonly used QSPRs and semi-empirical relationships when predicting seven partitioning and reactivity properties for chemicals registered in different chemical inventories. The results from this study show that more than half of the investigated chemicals are hydrophilic, lowly volatile and labile. The current QSARs can cover ~80% of chemicals in AD in terms of predicting  $K_{OW}$ ,  $K_{OC}$  and biotransformation half-lives. However,  $K_{OA}$  medians for chemicals in and out of AD differ by 6 orders of magnitude. In addition, current QSARs exhibit a narrower AD coverage of air hydroxylation rate constant and biodegradation half-lives which are criteria for persistent and long-range transport potential assessment. Thus, it is important to exercise caution when utilizing current QSARs for persistence and long-range atmospheric transport assessment. The AD coverage for semi-empirical relationship depends on how the AD is defined. For the first time, this presentation systematically informs academia and regulatory agencies on the AD of QSARs and semi-empirical relationships.

### 7.02.P-We555 Consolidated Octanol/Water Partition Coefficients: Combining Multiple Estimates from Different Methods to Reduce Uncertainties in log Kow

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The octanol/water partition coefficient (Kow) is a key parameter for assessing the fate and effects of chemicals. The log Kow can be determined experimentally, but more often it is calculated. Different methods can sometimes perform better and sometimes worse for different chemicals due to differences in the applicability domain related to, e.g., training set coverage and descriptor relevance. Variability in log Kow may be substantial and can be propagated in, e.g., quantitative structure-activity relationships (QSARs), *in vitro-in vivo*-extrapolations (IVIVE) and can bias the results. The present study was developed in the context of bioaccumulation assessment and aims to derive coherent log Kow estimates with known variability by: (1) estimating multiple log Kow values by different methods for diverse case study chemicals to exemplify their variability, (2) analysing the variability of log Kow estimates with regard to underlying methods and in terms of different chemical classes, and (3) recommending approaches to obtain reliable and robust log Kow estimates for hazard and risk assessment.

The case study chemicals include POPs, PCB, PAH, siloxanes, flame retardants, PFAS, pesticides, pharmaceuticals, fragrances, biocides, surfactants, UV-filter, plasticizer, antioxidants, etc. Up to 36 log Kow values per substance were obtained experimentally or estimated by different computational approaches such as group contribution (e.g. ClogP, EpiSuite), linear solvation energy relationships (LSER), read-across (e.g. Opera, ChemProp), deep learning neural networks (e.g. OCHEM), quantum chemical methods (e.g. COSMOtherm). Comparative analyses revealed that experimental and calculated log Kow can be highly variable (>1 log unit over the log Kow range from <0 to >8). No method is consistently superior. For most chemicals, variability of more than 1 log unit is the rule rather than the exception. Lack of (experimental) reference values for e.g. PFAS, siloxanes, surfactants, etc. compromises calibration of modelling results.

These results indicate that the search for the one and only best method for estimating log Kow may be futile. Instead, the mean of multiple log Kow values, including valid experimental data and estimates calculated using different methods, can provide reliable log Kow predictions. The benefits of the suggested approach are consolidated log Kow values that are scientifically credible, robust and reproducible, as well as cost efficient.

### 7.02.P-We556 How to build trust in the use of Artificial Intelligence for Chemical Risk Assessment?

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Risk assessment of chemicals is a time-consuming process and needs to be optimized to ensure all chemicals are timely evaluated and regulated. This transition could be stimulated by valuable applications of *in silico* Artificial Intelligence (AI)/Machine Learning (ML) models. However, implementation of AI/ML models in risk assessment is lagging behind the



scientific developments. Most AI/ML models are considered ‘black boxes’ that lack mechanistical explainability, causing risk assessors to have insufficient trust in their predictions.

In this study we stimulated the exchange of knowledge and information between computational modelers and chemical risk assessors. The aim was to work towards a common understanding of the possibilities of AI and the needs and requirements from a risk assessment perspective. We organized two workshops and collaboratively developed two predictive AI/ML models as case studies.

The results of this study highlight that trust is an essential factor towards regulatory adoption of AI/ML models and that it is not a static process, but needs to be built over time. We provide an overview of the elements of trust, including trust in technical (e.g., data quality, model uncertainty, etc.) and beyond-technical aspects (e.g., personal and societal beliefs), and highlight elements that are considered most important to build trust by risk assessors. These elements of trust should ideally be acknowledged in future developments to stimulate adoption of AI/ML models for regulatory purposes. In addition, we applied (most) of these recommendations in practice into two case studies: one on ecotoxicity for the prediction of Species Sensitivity Distributions, and one on human toxicity for the prediction of effects on development toxicity via the retinoic acid adverse outcome pathways.

This study highlights the importance of interdisciplinary activities to stimulate the adoption of state-of-the-art *in silico* models in a regulatory setting. Future efforts should further stimulate such collaborations between computational modelers and risk assessors, both at a national and an international level, as it will allow a more efficient and effective use of the already available data and knowledge.

#### **7.02.P-We557 Ecotoxicological Quantitative Structure-Activity Relationship Model Performances, and how they Underpredict Very Toxic Compounds**

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Estimations of the number of chemicals currently on the world market varies, but a recent study estimated over 350 000 chemicals and mixtures in the global inventories, and new chemicals are designed and made available every year. To keep up with the current pace of new chemicals, the use of high throughput predictive methods for ecotoxic effect assessment have been proposed, and quantitative structure-activity relationship (QSAR) models are commonly used for this purpose. In this study we have examined the predictive performance of three freely available ecotoxicological QSAR model frameworks: ECOSAR, Vega and T.E.S.T., for six endpoints commonly used in chemical regulation: acute and chronic toxicity for algae, daphnia and fish. We additionally examined the effect of ionizability, lipophilicity and experimental toxicity range of chemicals on prediction performance, through a scenario-based setup. For each scenario, model software and toxicity test, three performance metrics were calculated: coverage (ability to produce a reliable prediction), median error (median absolute log distance from prediction to experimental mean) and large deviations (predictions outside a factor 10 from experimental mean). Results indicate that there are differences in performance between models: Vega having the lowest coverage, while ECOSAR has the highest. For tests where T.E.S.T. can produce predictions, it has the lowest median error and lowest rate of large deviations. For other tests performance varies, and Vega performs better for chronic fish and daphnia, while ECOSAR has better performance for algae tests. Ionizability has very low impact on model performance for all models. For compounds with high logP values, the coverage of ECOSAR goes down (by 15 to 75 percentage points), and all models perform worse for acute daphnia and fish (most data rich endpoints). Toxicity range analysis show a troubling trend: QSAR models systematically underpredict toxicity for high toxicity compounds and overpredict toxicity for low toxicity compounds.

#### **7.02.P-We558 Implementing in silico approaches for human toxicity prediction in the Dutch drinking water sector**

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Over the years, an increasing number of chemicals and toxicological endpoints have been receiving attention in the (Dutch) drinking water sector. For many of these chemicals, little is known about their toxicity as it is time consuming and expensive to conduct *in vitro* and especially *in vivo* toxicity experiments. *In silico* approaches are useful in estimating hazards associated with such chemicals. Over the years, KWR Water Research has produced QSARs based on both linear regression analysis and Random Forest analysis to predict the toxicity, as measured in a large number of *in vitro* assays, of chemicals based on chemical structure and molecular descriptors. To gain insight into the toxicity of existing and emerging chemicals, the complete ToxCast dataset was downloaded. AC<sub>50</sub> data (in  $\mu\text{M}$ ) from this database were formatted based on a set of criteria. The resulting data consisted of 5,114 chemicals, and for 603 endpoints for *in vitro* assays. Toxicity (AC<sub>50</sub>) was predicted using both Random Forest and linear regression models, based on chemical structure and molecular properties. These models were evaluated by looking at the model performance of a test dataset.

Overall, large variation in the predictive power of both models were observed across assay endpoints and modelling techniques. Furthermore, different structural elements and molecular descriptors of chemicals drive toxicity in different assay endpoints. In general, linear regression models performed better than Random Forest models. However, this does not necessarily imply that some endpoints may be easier to predict as there may be multiple explanations (i.e. the datasets may have been too small, the models may contain a large proportion of irrelevant structural elements or the AC<sub>50</sub> data itself is expected to be noisy). The studies presented here aimed to gain a deeper understanding of the methodology of *in silico* prediction of toxicity using the ToxCast database, allowing the signaling of new and potentially hazardous chemicals that may emerge in the aquatic environment. Structural elements and physicochemical descriptors of chemicals for a subset of *in vitro* assay endpoints did accurately predict AC<sub>50</sub>s and AC<sub>50</sub> classes (i.e. 'low', 'medium', 'high'). In future research we foresee the development of a tool to predict toxicity classes, rather than exact toxicity (AC<sub>50</sub>) values for particular subsets of endpoints, which may aid in the first prioritization of chemicals.

#### **7.02.P-We559 Are We Justified in Modeling Human Exposure to Chlorinated Paraffin Mixtures Using the Average Properties of Congeners and Homologues?**

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Human exposure to chlorinated paraffins (CPs) is a significant environmental and health concern due to the potential for persistence, bioaccumulation, and toxicity associated with these compounds. However, assessing human exposure to such mixtures is still challenging, given the facts that (i) CP mixtures consist of millions of components, making it difficult for existing analysis methods to separate and quantify them accurately and consistently; (ii) "complex" exposure modeling that accounts for each component is currently time- and resource-intensive. In this presentation, we explore whether and at what level (e.g., congener or homologue groups) we can aggregate CP mixtures by using average physicochemical properties in human exposure modeling. The central question guiding this exploration is the influence of physicochemical properties on human exposure to CP mixtures. Relying on properties predicted for individual CP isomers, we aim to answer this question by comparing exposure predictions obtained through a "complex" method utilizing isomer-specific properties against "simplified" methods that leverage average properties of congeners, homologues, or Short-/Medium-/Long-Chain CP groups. Our results reveal a broad range of physicochemical properties across CP mixtures and their dependence on molecular structures. These variances translate into an extensive disparity in whole-body concentrations predicted for different isomers, spanning ~11 orders of magnitude. CPs with 13-19 carbons and 6-10 chlorines exhibit the highest relative human exposure potential, primarily owing to their moderate to high hydrophobicity and slow environmental degradation and biotransformation. Except for highly volatile components, far-field exposure is dominant for most CP components. Despite the large variance in properties and human exposure potential of CP isomers, our work justifies that using average properties of congener, homologue, or S/M/LCCP groups yields results that are consistent with those derived from isomer-based modeling. This presentation not only enhances our understanding of human exposure to CPs but also provides insights into how *in silico* modeling approaches can contribute to efficient and practical human exposure assessment strategies for complex chemical mixtures.

#### **7.02.P-We560 EAS-E Suite: A comprehensive web-based platform to integrate *in vivo*, *in vitro* and *in silico* data for chemical safety and sustainability**

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The modernization of chemical screening and risk assessment is confronted with the need to address data gaps in hazard and exposure. New Approach Methods (NAMs), such as *in vitro* bioactivity data, *in silico* approaches (e.g., Quantitative Structure Activity Relationship – QSAR), and high-throughput toxicokinetics (HTTK), have emerged as promising solutions. However, the effective application of NAMs for decision-making requires a deep understanding of their relevance, reliability, applicability domains (AD), as well as the integration of the different approaches in a coherent weight of evidence framework.

Exposure and Safety Estimation (EAS-E) Suite is a web-based platform designed to bridge the gap between advancing scientific methods and chemical safety assessment. EAS-E Suite serves as a hub for gathering, harmonizing, and integrating diverse chemical data, QSARs, environmental fate and exposure models. It houses a curated database containing experimental physical-chemical properties and toxicokinetic data. Additionally, it provides predictions from models like EPISuite, OPERA, IFSQSAR, and QSARINS-Chem, allowing for a consensus approach. Each QSAR prediction comes with additional information (e.g., AD) in compliance with the recently published OECD QSAR Assessment Framework. Moreover, predictions can be directly generated for chemicals not in the database using SMILES notation.

The databases and QSARs provide information to parameterize a suite of models to generate environmental fate and exposure predictions. EAS-E Suite includes models to estimate mode of entry and emission rates (i.e., CiP-CAFE) as well as regional, point source and indoor environmental fate, bioaccumulation, and exposure (e.g., RAIDAR, RAIDAR-Point Source, RAIDAR-ICE). A generic one-compartment physiologically based kinetic model that can be parameterized for different organisms (e.g., human, rat, fish) is implemented to allow HTTK simulations. All the models are harmonized and integrated to

allow the estimation of aggregate exposure in the PROTEX-HT model. A preliminary version of sensitivity and uncertainty analysis is included in the simulations to investigate how uncertainties in input parameters propagate through the modeling process, improving the interpretability of the results. With entry of only chemical structure, the system can select the best values to parameterize the different models and provide critical information for holistic ecological and human health assessments.

#### **7.02.P-We562 Developing Sustainable Alternatives for Persistent, Mobile, and Toxic Chemicals: A Safe and Sustainable by Design (SSbD) Approach**

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Persistent, mobile and toxic (PMT) as well as very persistent and very mobile (vPvM) substances threaten drinking water quality and ecosystem health. PMT/vPvM substances do not readily biodegrade in the environment, can travel long distances and are difficult to remove with current water treatment technologies. PMT chemicals are also toxic to humans and/or biota. Despite current regulatory systems to manage the risks of chemicals, issues with chemical safety persist and concerns related to PMT/vPvM chemicals are pertinent. This highlights the need for additional measures to protect water resources, as well as human and environmental health from adverse impacts arising from exposure to these substances.

Recognised as highly effective methods for reducing environmental and human health hazards, are upstream preventative approaches. In particular the Chemicals Strategy for Sustainability acknowledges the Safe and Sustainable by Design (SSbD) approach. SSbD includes safety and sustainability considerations at the beginning of the chemical innovation process alongside functionality considerations, to aid in the selection of less hazardous chemical alternatives for a given application. The use of screening and predictive models can bridge the gap arising from limited experimental data on persistence, mobility and toxicity for most chemicals, to prioritise where further testing is needed. However, well-elaborated tools for the selection of SSbD alternatives for PMT chemicals that fulfil desired and essential functions in a given application are still missing.

The project presented here aims to develop a simultaneously concrete and generally applicable workflow for the selection of SSbD alternatives for PMT/vPvM substances that is applied in consultation with different stakeholders. Publicly available databases will be used to select relevant PMT/vPvM priority substances, based on problematic applications in consumer products. Large sets of potential chemical alternatives for these substances will be generated *in silico* and later screened for PMT/vPvM properties, as well as properties relevant to sustainability, and functionality. Existing databases and QSAR models, (e.g. EPISuite, BIOWIN and VEGA) will be used. Results of *in silico* screening will be complemented by experimentally generated data. Through this project we aim to make SSbD more mainstream, generally applied and well-accepted in daily practice.

#### **7.02.P-We563 Support of persistence assessment under REACH and CLP using *in silico* predictions**

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With the introduction of hazard classes for persistent chemicals under Regulation (EC) No 1272/2008 (Classification, Labelling and Packaging of substances and mixtures - CLP), companies and European Member State authorities face the challenge of assessing the potential for persistence of a high number of substances in a complex regulatory environment, and within a short period of time.

More than 2000 substances registered under Regulation (EC) No 1907/2006 (Registration, Evaluation, Authorisation and Restriction of Chemicals - REACH) with a tonnage > 10 t/a are considered “potentially persistent” since they either fail the criteria for ready or inherent biodegradability, or due to a lack of suitable biodegradation data. In such cases, higher tier simulation biodegradation studies (i.e., OECD 307, 308, and 309) are required to derive biodegradation half-lives. Simulation studies are very expensive, technically challenging and have variable outcomes due to the considerable influence of environmental parameters and experimental design. For substances of unknown and variable composition (UVCBs), the experimental design is specifically challenging. It may even require several studies to run consecutively, or may not be feasible at all. Moreover, limited laboratory capacity and the relatively few knowledgeable experts in this field further limit the availability of sufficient higher tier experimental data for persistence assessment.

In addition to assessing the persistence of the parent substance, the persistence of relevant transformation / degradation products must also be considered (according to CLP and REACH). Furthermore, appropriate identification of metabolites by simulation testing of biodegradation is often missing.

Here we describe the challenges which authorities and companies face in coming to a conclusive persistence assessment under CLP and REACH. We present how *in silico*-generated predictions can support persistence assessments based on biodegradation screening test results. Furthermore, we present how the generation of potential transformation products may be predicted, and their respective hazard profile quickly assessed using *in silico* screening techniques. The proposed strategy represents a fast, cost-effective alternative to higher tier simulation testing.

### **7.02.P-We564 Computational Characterization of Sulfate-Reducing Bacteria Inhibitors to Overcome Methanogenic Competence and Optimize Green Biogas Production**

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Microorganisms play a crucial role in wastewater treatment plants (WWTPs). Such is the case of methanogenic archaea, which are able to break down organic matter and remove pollutants from wastewaters and sludges produced during its treatment, producing methane as a by-product. WWTPs could use this methane as a renewable energy source, instead of fossil energy, committing to the circular economy. However, sulfate-reducing bacteria (SRB) compete with methanogenic archaea for the same substrate in anaerobic conditions, thus reducing the efficiency of methane production and generating H<sub>2</sub>S, a harmful gas that can also have adverse consequences on environment. Therefore, it is of utter interest to study the possible inhibition of SRB to both optimize the methane production and to reduce H<sub>2</sub>S emissions.

In the present study we have employed different approaches to find potential SRB inhibitors. From one side, we have developed Quantitative Structure-Activity Relationship (QSAR) models and used them to perform virtual screening of COCONUT, a database of natural substances. From other side, different databases (KEGG, BRENDA, PDB and PubChem) were manually explored to find specific inhibitors. Finally, one interesting candidate from the virtual screening and an analogous were identified with similar predicted activity. All the candidates found with the three approaches were combined and a final step consisting of ecotoxicological predictions was implemented to remove all the possible ecotoxic molecules.

The QSAR models' metrics were acceptable for predicting the inhibitory activity against methanogenic archaea and SRB. The QSAR modelling and the exploratory approaches yielded 9 and 11 candidates respectively. Altogether, 20 potential inhibitors were obtained and only 4 were selected, based on commercial availability and several properties, to carry on with the experimental validation, which is currently ongoing.

The potential candidates identified might allow the reduction of the substrate competence between methanogenic archaea and SRB; therefore, methane production will potentially increase and could supply energy to the same WWTP. Consequently, this will lower the greenhouse gases by consuming self-produced biogas in WWTPs.

### **7.03.P One Health Approach: PFAS Exposure in Wildlife and Shared Health Risks Across Species Including Humans**

#### **7.03.P-Tu538 Striped Dolphins as Bioindicators: Tracing Per- and Polyfluoroalkyl Substances Pollution in the North West Mediterranean Over Three Decades**

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Poly- and Perfluoroalkyl Substances (PFAS) constitute a class of global and persistent pollutants with known biomagnification capacity, warranting their investigation as threats to the conservation of species such as marine mammals. This study explores striped dolphins as bioindicators of PFAS pollution in the North-Western Mediterranean Sea, evaluating biomagnification rates and temporal trends of PFAS over three decades (1990-2021) in stranded specimens. This study focused on evaluating PFAS biomagnification in mature striped dolphins in the NW Mediterranean and tracking PFAS concentration trends from 1990 to 2021.

PFAS analysis was conducted on striped dolphins' digestive content and liver, involving liquid-solid extraction and analysis using UPLC-MS/MS, identifying and quantifying 19 PFAS compounds. Thirteen PFAS compounds were identified in the digestive content, while liver samples revealed the presence of 17 PFAS compounds. Linear perfluorooctanesulfonic acid and perfluorooctanesulfonamide emerged as the predominant compounds in all samples, closely trailed by long-chain carboxylate acids. Long-chain PFAS exhibited greater biomagnification rates than short-chain PFAS, suggesting potential health concerns for striped dolphins. These results highlight the risks posed by the accumulation of long-chain PFAS, emphasizing the need to study PFAS profiles and their impact on vulnerable species.

Temporal trends showed an increase in concentrations of most long-chain PFAS from 1990 to 2004-2009, stabilizing during 2014-2021. This stabilization may signify the impact of regulatory measures and industry initiatives aimed at reducing PFAS pollution. However, analysis revealed that half of the digestive content samples exceeded Environmental Quality Standards from the Water Framework Directive, underscoring the need for ecological risk assessments and stricter regulations, and an emphasis on risk reduction and shared health risks across species.

In conclusion, this research contributes to the understanding of PFAS biomagnification in striped dolphins, revealing persistent contamination in the North-Western Mediterranean Sea and highlighting the interconnected health concerns of marine

ecosystems and humans. In turn, this study builds up on the current urgency for continued research and stringent regulations able to address the lasting threat of PFAS in the environment.

### **7.03.P-Tu539 A comprehensive analysis of PFAS change points in humans and the environment using archived samples from Germany**

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Per- and polyfluoroalkyl substance (PFAS) contamination is a widespread global problem that has a proven negative impact on the terrestrial, freshwater and marine environment. Due to their hazardous properties and high risks to humans and the environment, certain PFAS such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were voluntarily phased out by their main manufacturer in the early 2000s and have been regulated by the European Union since 2006 and 2020 respectively. Global restrictions were introduced by the Stockholm Convention in 2009 for PFOS, in 2019 for PFOA and in 2020 for perfluorohexanesulfonic acid (PFHxS). While human and wildlife exposures are well characterised for the conventional target PFAS, little is known about time trends and the assessment of the effectiveness of regulatory changes. Previous analyses of suspended particulate matter (SPM) from Germany showed a log-linear decrease since 2005 for both, perfluorosulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs). As the SPM sampling started only after the main PFAS were phased out, we performed a comprehensive time trend analysis of samples from the German Environmental Specimen Bank going back to the 1980s and early 1990s. These samples covered human plasma as well as samples from the terrestrial (deer liver), freshwater (bream liver and muscle) and marine environment (herring gull eggs). The aim of the study was to statistically evaluate the change points (CPs) of exposure and quantify the changes before and after the CPs to assess the effectiveness of the above measures. The CPs were estimated using the R-package “segmented” by two linear regression splines with the CP representing the joint point. The analysis demonstrated remarkably similar CP estimates across human and environmental matrices. The results showed that the maximum concentrations of PFOS were already reached before (late 1990s) the announcement of the phase-out by their main manufacturer. These results are similar for all tested PFSAs and indicate an earlier shift to PFAS alternatives than previously expected. For PFCAs, CPs were generally later (mid-2000s), possibly due to the delayed regulatory action on PFCAs and associated preceding production shifts. In general, regulatory actions were introduced ~10-15 years after the respective CPs, which demonstrates the need for earlier regulatory actions to effectively protect humans and the environment.

### **7.03.P-Tu540 Do Per- and Polyfluoroalkyl Substances (PFASs) and Other Pollutants Threaten Shorebirds Inhabiting Artificial Wetlands?**

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The prime threats perceived to contribute to the global decline of shorebirds include climate change and habitat loss, while the role of pollution in these declines has been less investigated. Whereas the rate of destruction of natural wetland habitats has been particularly rapid over the past decades, artificial wetlands, including wastewater treatment plants, have seen an increase over this time and provide alternative habitats for a wide range of bird species. These new habitats, in turn, pose a pollution risk to wildlife.

We compared exposure to pollution by 15 per/polyfluoroalkyl substances (PFASs) and 15 elements along with prevalence of avian influenza, oxidative stress and local survival in two long-distance migratory shorebird species, curlew sandpipers (*Calidris ferruginea*) and red-necked stints (*Calidris ruficollis*). Birds were sampled for blood from 2011-2020, at two contrasting habitats on their Victoria, Australia non-breeding grounds: a natural wetland on Western Port Bay and a putatively more polluted artificial wetland at Melbourne's Western Treatment Plant (WTP), the latter of which processes the waste of approximately 5 million people.

Blood pellet concentrations of both carboxylate PFASs and sulfonate PFASs were found to be significantly higher at the Western Treatment Plant (carboxylate PFASs at WTP median: 15.7 ng/g, range: <0.01-107 ng/g; natural wetland: 2.05 ng/g, <0.01-62.3 ng/g - sulfonate PFASs at WTP median: 65.3 ng/g, range: <0.01-804 ng/g; natural wetland: 4.86 ng/g, <0.01-26.9 ng/g). Nevertheless, concentrations were generally below reported health risk threshold values.

Avian influenza prevalence was higher at the natural wetland, while seropositivity (representative of prior infections) was higher at the WTP. We also measured higher blood o,o'-dityrosine (an indicator of protein damage) at the WTP. No significant differences were found for adult survival, but survival of immature birds at the WTP appeared to be lower than those at the natural wetland.

Our findings showed that PFASs from wastewater treatment plants indeed contaminate the birds inhabiting the region. However, concentrations in most individuals were lower compared to health risk thresholds. These relatively low concentrations, together with limited other significant differences in pollution and health indicators, tentatively suggest that appropriately managed wastewater treatment wetlands may provide an alternative habitat to these migratory species.

### **7.03.P-Tu541 Gene Expression of Mitochondrial Antioxidant Enzymes in a Freshwater Fish Species (*Squalius Cephalus*) Exposed to Environmental Concentrations of Pfas in the Veneto Region.**

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Since its discovery in 2013, the Veneto region has been affected by one of the most extensive PFAS contaminations in the world, originated by a fluorochemical plant in Vicenza's province, active since the late 1960s. PFAS are highly persistent in the environment. Exposure to environmental stressors, like pollutants, can affect animals' physiology, in many cases inducing a response from the organism at the level of the antioxidant system. In particular, PFAS can bioaccumulate and biomagnify along food webs, affecting ROS production at the molecular level, hence unbalancing the cellular redox equilibrium. This may result in cell death, DNA damage, altered enzymatic function, and consequent oxidative damage to the entire organism. Therefore, variations in the content and the activity of antioxidant enzymes are useful as biomarkers for oxidative stress caused by contaminants in marine organisms, enabling evaluation of their potential risk for aquatic ecosystems and biota. The aim of the present study is to evaluate the possible effects of chronic exposure to different environmental concentrations of PFAS and the induced physiological responses in a freshwater fish species: *Squalius cephalus* (Linnaeus, 1758) which populates the Veneto rivers. In particular, some molecular and biochemical analyses, which consist in evaluating some oxidative stress biomarkers, were carried out in the kidney. Ten specimens of *Squalius cephalus* were sampled by electrofishing from three rivers in Vicenza (Veneto, Italy) characterized by three different levels of PFAS contamination. First, expression analyses were performed at the transcriptional level of genes coding for four mitochondrial isoforms (sod2, gpx4, prdx3, prdx5) of antioxidant enzymes, evaluating the mRNA accumulation by real-time qPCR. Then, two indicators of cellular damage were measured: Lipid Peroxidation and Advanced Oxidation Protein Products (AOPP) formation. To summarize the results, we can observe a qualitative response which is reflected in the involvement of different components of the antioxidant system, but also quantitative in relation to different levels of environmental contamination. Exposure to PFAS increases the rate of ROS formation, both of O<sub>2</sub><sup>-</sup>, as demonstrated by the increase in sod2 expression, and of H<sub>2</sub>O<sub>2</sub>, as demonstrated by the activation of the scavenger enzymes of this ROS (gpx4, prdx3, prdx5).

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### **7.03.P-Tu542 Mapping PFAS Distribution in Flanders, Belgium: Results of an Extensive Aquatic Monitoring Campaign**

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In recent years, public awareness has increased regarding the presence of PFAS (per- and polyfluoroalkyl substances) in the environment. Until recently, little information was available on the occurrence of PFAS in Flanders. Therefore, a large-scale monitoring campaign was initiated by the Flanders Environment Agency (VMM) in 2022 to map the distribution of PFAS contamination in this region. Wastewater, surface water, sediment, fish, and groundwater were analyzed from both suspected and non-suspected sites for 43 PFAS (49 for groundwater). Although many PFAS were below the detection limit, at least one PFAS was detected in more than 90% of the wastewater, surface water and fish samples. For sediment and groundwater, this was around 30% of the samples. The median  $\Sigma$ PFAS concentration in industrial wastewater ranged between 50 and 570 ng/L, while effluent from sewage treatment plants (STP) had a median of 50 ng/L. Surface waters showed a similar median concentration compared to the STPs, namely 45 ng/L  $\Sigma$ PFAS, while the median groundwater concentration was about 5 times lower (8.7 ng/L  $\Sigma$ PFAS). The median concentration in sediment was 1.2  $\mu$ g/kg dw  $\Sigma$ PFAS. These results were in the same order of magnitude as those in neighbouring countries and regions when comparing amongst highly urbanized or industrialized areas. Between matrices, some clear differences in PFAS profiles could be observed. Distinct fingerprints could be assigned to wastewater based on industrial sector. However, sector fingerprints were not distinguishable in surface water due to the diffuse sources in receiving waters. Overall, the water compartments (wastewater, surface water and ground water) showed similar PFAS profiles, although when including the confined aquifers, groundwater showed a different profile. In sediment and fish, long-chain PFAS and precursors were more prevalent, in contrast to short-chain PFAS in water. These differences are likely due to the physico-chemical characteristics of individual PFAS. Measured concentrations were compared to available thresholds for environmental and human health, showing most exceedances for surface water and biota, depending on the selected threshold value. Altogether, this report revealed that PFAS can be widely detected in the Flemish environment. The study outcome allows for decision-making by the Flemish government as part of the PFAS action plan and will aid to prioritize future sampling efforts.

### **7.03.P-Tu543 Toxicity assessment of per- and polyfluoroalkyl substances (PFAS) with varying chain lengths on *Daphnia magna* and *Allivibrio fisheri*.**

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The LIFE CAPTURE project aims to investigate the characteristics and behaviour of PFAS and identify innovative remediation solutions for these persistent soil and groundwater contaminants. We seek to devise a practical approach for assessing the risks and effects of PFAS, utilising standardised bioassays (ecotoxicity tests) to ensure ease of implementation

and economic viability. Special attention is paid to risk assessment for mixtures of PFAS and methods for assessing risk reductions achieved by remediation.

In this extensive project, we present the first results obtained on two standard organisms, namely *Daphnia magna* and *Aliivibrio fischeri*. For all the tested congeners, *Aliivibrio fischeri* showed greater sensitivity than *Daphnia magna*. Moreover, survival rates and light emission inhibition decreased with increasing chain length. For both model species the most toxic potential is found for the shortest chain PFASs (3 and 4 C). The confirmed pattern of additive concentrations is shown in the bioassay data obtained from test mixtures. Currently, PFAS toxicity is an active area of research since scientists aim to understand the mechanisms behind these effects in addition to their impact on aquatic ecosystems. These studies improve our understanding of how PFAS pollution can impact freshwater organisms, enabling the development of regulations and management plans to reduce harm to the environment. However, the overall comprehension of the toxicity of PFAS compounds, varying from shorter to longer chain ones, is still an area of ongoing research. Environmental conditions, interactions among different PFAS compounds, and their combined effects may also influence their overall impact on ecosystems. Environmental agencies evaluate continuously the toxicity of PFAS substances in order to formulate guidelines and regulations to mitigate their environmental and health effects.

### **7.03.P-Tu544 Hepatic concentrations of per- and polyfluoroalkyl substances (PFAS) in dolphins from south-east Australia: Highest reported globally**

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Per- and poly-fluoroalkyl substances (PFAS) are a diverse group of over 6,000 stable, man-made compounds found in a wide array of household and industrial products, creating a ubiquitous environmental presence. This study addresses the critical knowledge gap regarding PFAS contamination in Australian aquatic environments, focusing on marine mammals.

We conducted an investigation of PFAS concentrations in four dolphin species stranded along the south-east Australian coast. Here we report the highest concentration of any Cetacea globally, in the critically endangered Burrunan dolphin. Specifically, populations in Port Phillip Bay (median 9750 ng/g ww, n = 3) and Gippsland Lakes (median 3560 ng/g ww, n = 8) recorded concentrations 50–100 times higher than other species in the study, including common bottlenose dolphin (50 ng/g ww, n = 9), Indo-Pacific bottlenose dolphin (80 ng/g ww, n = 1), and short-beaked common dolphin (61 ng/g ww, n = 12). Also included in the results is the highest reported individual ΣPFAS (19,500 ng/g ww) and Perfluorooctane sulfonate (PFOS, 18,700 ng/g ww) concentrations, at almost 30 % higher than any other Cetacea reported globally.

PFOS was found to be the predominant PFAS compound, exceeding tentative critical concentrations in 42% of all dolphins and 90% of the endangered Burrunan dolphin. Notably, we identified for the first time novel and emerging PFAS; 6 perfluoro-3-methoxypropanoic acid (PFMPA), perfluoroethylcyclohexane sulfonate (PFECHS), 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA) and Perfluorobutane sulfonamide (FBSA), in southern hemisphere marine mammals, with high detection rates across the dataset.

This research provides the first documentation of PFAS in the tissues of multiple Cetacea species in the Australasian region. These findings emphasize the global significance of high PFAS concentrations in inshore dolphins and contribute vital baseline knowledge for understanding PFAS exposure and bioaccumulation in the coastal environment of south-east Australia.

### **7.03.PC One Health Approach: PFAS Exposure in Wildlife and Shared Health Risks Across Species Including Humans**

#### **7.04 Safe and Sustainable by Design Advanced Materials: What Does It Take?**

**7.04.T-01 Comparative Analysis between Tier 1 SSbD (Safe and Sustainable by Design) approach and Early4AdMa**  
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The Chemicals Strategy for Sustainability (CSS) and Zero Pollution Action Plan call for a transition towards a Safe and Sustainable by Design (SSbD) approach for chemicals and emerging materials. This has led to the EC's Recommendation for establishing a European assessment framework for SSbD chemicals and materials. To facilitate the implementation of this framework the H2020 SUNSHINE project developed a tiered SSbD approach, while at the same time a consortium composed by RIVM (NL), BfR (DE), BAuA (DE) and UBA (DE) proposed the Early4AdMa early warning system. The SUNSHINE SSbD approach aims to provide support to industries in developing SSbD strategies for specific advanced materials and products incorporating them. The Early4AdMa system offers a structured methodology for pinpointing possible safety, sustainability, and regulatory issues that may arise from emerging advanced materials. The two approaches are similar, and in order to assist users in selecting the system that is most suited to their needs, a comparative analysis of the two methods was carried out, and it was performed in the frame of the OECD Working Party on Manufactured Nanomaterials and specifically in the Steering Group on Advanced Materials. The analysis aims to identify similarities, differences, strengths, weaknesses and

targeted users. The two methods are composed of a questionnaire which questions were classified in common aspect categories such as safety, environmental, economic and social sustainability, and functionality. An additional category, applicability to regulatory frameworks, was relevant only for Early4AdMa. The results showed that the two approaches are highly complementary. Early4AdMa is instrumental in pinpointing safety and sustainability issues that may not be covered by current regulations and is therefore aimed for application by regulators. On the other hand, the SUNSHINE approach is tailored to identify hotspots of safety and sustainability concerns along the lifecycles of specific materials/products on a case-by-case basis to support SSbD decision making by industries, including SMEs. Thus, the two approaches can be synergistically applied to facilitate implementation of the SSbD framework by different stakeholders: SUNSHINE is better tailored to industries and consultants, while Early4AdMa is targeted at policy makers, the standardisation community and regulators. Further comparison on the output of the two approaches will be performed by assessing two cases

#### **7.04.T-02 Holistic Design Methodology for Circularity adapted to the SSbD framework**

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On the way to determine a path to avoid or reduce harmful effects on human health and the environment from materials and products throughout its complete life cycle, design plays a key role in innovation and business strategy settings. The development of the Safe and Sustainable by Design (SSbD) framework also highlights the relevance of design as a key driver to ensure more safe and sustainable chemicals and materials considering the three pillars of sustainability (environment, economic and social).

In this research, a SSbD eco-design methodology is presented. The bestowed methodology ensures the proper implementation of the framework, enhancing the analysis through continuous improvement at all stages, thus aligning with the various SSbD design principles. This approach stems from the integration of EURECAT's registered eco-design methodology and the above mentioned SSbD framework.

The SSbD eco-design methodology aims to be a holistic and iterative process that will provide an overall vision of the final product (chemical or material) and a set of priorities. Four key successive steps are defined following the Plan-Do-Study-Act (PDSA) approach: 1) Identification of Hotspots from the five steps described on the framework, 2) Eco-design SSbD principles and strategies definition and set up, 3) Re-adaptation of technological procedures to the selected strategies and 4) Definition of concrete eco-design actions, for which all relevant stakeholders are invited to participate in order to obtain feedback from the different perspectives and experience to define specific actions and to prioritize them through the use of interactive platforms and the Analytic Hierarchy Process (AHP) methodology.

In conclusion, the application of the SSbD eco-design methodological approach ensures that the future chemicals or materials developed under this framework will have a lower hazard and risk impact in their conception and applicability while simultaneously improving its sustainable profile. Through its iterative process, a continuous improvement can be obtained without compromising technical performance, and the level of implementation of the actions is subject to the project development.

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#### **7.04.T-03 Life Cycle Safety and Sustainability Assessment of Innovative Solutions for Art Restoration**

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The conservation of Cultural Heritage (CH) necessitates continuous interventions, that can be harmful to human health, particularly for operators and curators, and at the same time impactful to the environment. Coping with these issues, GREENART (GREEn ENdeavor in Art ResToration) project, funded by the HORIZON Europe Programme, proposes new advanced materials and chemicals to conserve CH (i.e., cleaning systems, protective coatings and consolidants, packaging and sensors). As part of the project, a Life Cycle Safety and Sustainability Assessment is planned to provide step-by-step guidance to product developers to ensure the development of safe and sustainable innovative solutions. The research employed the sustainability framework established in the H2020 NANORESTART project, which implements the Safe by Design (SbD) concept, and integrates it with the "Safe and Sustainable by Design chemicals and materials" framework by the Joint Research Center (JRC). The initial step, focusing on hazard assessment, served as a guide for safety evaluations of GREENART chemical solutions. This first evaluation was performed according REACH and CLP regulations to look at the intrinsic properties of the chemical or material in order to understand its hazard profile before further assessing the safety during use. By combining Step 2 of the NANORESTART Framework and Step 1 of the SSbD Framework, all formulation ingredients were assessed for Human Health (H), Environmental (ENV) and Physical (P) Hazards, by consulting Safety Data Sheets and/or the ECHA dossier. Then, all ingredients were classified (Levels 0 to 3) according to the SSbD criteria for assessing intrinsic hazard properties. These initial results were communicated to the formulation developers along with observations for each



SSbD hazard level. Finally, a self-classification of the mixture in accordance with CLP guidance was performed. Based on the outcomes of this initial phase, the developers of the product have the option to modify the original formulation to minimize any potential risks, or they can choose to eliminate it altogether, if functionality cannot be preserved. This work will be completed by safety evaluation for the production and application phases and, finally, by Life Cycle Assessment (LCA) and Life Cycle Cost (LCC). This work is expected to contribute to the development of criteria for chemicals and advanced materials to support the application of the SSbD framework to the CH sector.

#### **7.04.T-04 Application of the SSbD framework to biocidal nanocoatings: gaps and steps towards its implementation**

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The Safe and Sustainable by Design (SSbD) Framework has the aim to facilitate the design and development of chemicals and materials to be safe and sustainable through research and innovation initiatives. The framework considers the intrinsic hazards of a chemical or material, environmental and human safety during production, use and disposal as well as environmental sustainability through life cycle assessments. The result of the assessments provides a score that indicates the level of SSbD. The SSbD framework is currently in the development and testing phase, and presently, there are no specific approaches available for specific groups of chemicals or materials. In particular it is unclear how functional and societal benefits can be included within the SSbD Framework. Biocides, for instance, are being used for the protection of living organisms against infectious diseases. Nevertheless, their impact may extend beyond controlling the intended harmful organisms, potentially affecting humans and non-target organisms in the environment. Biocides are also being promoted to achieve several United Nations Sustainable Development Goals, however, it is so far not possible to consider this within the SSbD. Thus, due to the dual nature of biocides—offering benefits and carrying negative effects caused by their nature as being toxic to target organisms—a specific SSbD approach considering their functionality is crucial for evaluating their safety and sustainability alongside societal benefits. In this study, we developed criteria that may be applicable to the SSbD framework for assessing the functionality-related benefits of biocidal products. Our study covers: *i*) assessing the data and methodological challenges to apply SSbD to biocidal products, and providing recommendations, *ii*) suggesting methodological approaches that can be integrated into the SSbD framework to account the benefits of the biocides, *iii*) addressing the challenges of applying SSbD to the early design stages of biocidal nanocoatings, and providing possible approaches, *iv*) presenting a case study demonstrating the application of the SSbD framework to biocidal nanocoatings for public transport use.

#### **7.04.P Safe and Sustainable by Design Advanced Materials: What Does It Take?**

##### **7.04.P-We565 Safe and Sustainable-by-Design – A guidance to unleash the transformative power of innovation**

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Cefic and its members have defined, in their previous reports from October 2022 and April 2023, Safe and Sustainable-by-Design (SSbD) as an iterative process guiding innovation and the placement on the market of solutions that are safe, and deliver environmental, societal, and/or economical value through their applications. In scope are new chemicals, materials, products, processes and services, as well as the re-design of existing ones.

The 3rd report proposes guiding design principles for a selected set of safety and sustainability considerations, dimensions or **criteria** to be assessed at the level of product-application combination in a stage-gate-like approach during innovation.

The basic principle when innovating to improve the functionality and performance of chemicals, materials, products, processes or services, is the aim to significantly improve performance in at least one of the dimensions of safety and sustainability without significant negative impacts in any of the other dimensions, compared to the incumbent solutions.

As a minimum, a sound implementation of “**Safety**” shall be applied by a risk-based assessment considering the hazard, use and exposure in line with REACH and anticipating future regulatory changes. But in applying SSbD, the chemical industry has the ambition to go *beyond* that legally fixed minimum requirement and go for continuous reduction of toxicological risks for humans and the environment especially for consumer use and considering end-of-life and circularity aspects.

The “Sustainability” assessment as an integral part of the innovation process shall cover the life cycle of a product-application-combination. All assessments shall, as a minimum, cover focus dimensions deemed of high importance to reach the Green Deal objectives. Additional sustainability contributions may be considered.

##### **7.04.P-We566 Towards Regulatory Preparedness of Advanced Materials: the OECD Early4AdMa System**

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Advanced materials, may offer many economic advantages and some of them could help to deliver solutions to societal problems such as the need to reduce reliance on fossil fuels. However, some advanced materials may pose risks to human health or the environment, as well as having the potential to create other unintended sustainability issues. Further, it is not clear whether the existing (inter)national legislation and test methods for chemicals (including nanomaterials) are 'fit-for-purpose' to cover the potential issues posed by advanced materials. Early identification of those issues is a first step for regulators (and innovators) to become prepared for possible action. Indeed it enables early anticipatory actions like pointing out to information gaps, concerns, and regulatory needs. Thus, providing the basis for relevant research and the development of guidance, test systems or adaptation of legislation to prevent possible negative impacts of newly developed materials. In this presentation, an early awareness and action system (Early4AdMa) that was developed within the OECD is presented. The system aims to systematically identify potential human health, environmental, sustainability and regulatory issues of advanced materials. The system is composed of two tiers. Tier 1 is a broad screening assessment. Tier 2 is a detailed assessment of potential issues of a selected advanced materials based on four sets of questions. Based on the identified issues in Tier 2, suggestions for follow-up action (e.g. research and/or regulatory activities) are identified. These can be communicated to regulatory decision makers, policy makers and risk assessors so that potential issues can be addressed early on. Further details on the different steps, scope and limitations, and notes on how to use the system will be presented. In addition, in the presentation, other activities towards regulatory preparedness of advanced materials that are being conducting within the OECD will be presented and discussed, amongst other the collaboration with the OECD Steering Group on the Safe-and-Sustainable-Innovation-Approach (SSIA). The Early4AdMa system may help identify potential issues of newly developed materials, and thereby, aims to contribute to the delivery on solutions for societal and environmental challenges via application of advanced materials.

#### **7.04.P-We567 Implementing Safe and Sustainable by Design (SSbD) approach in the design of biobased, recyclable multifunctional composites for automotive and aeronautical sectors**

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Advanced Materials (AdMs) are key to enable the green transition and are strategic for the transport sector, where a key driver to improve energy consumption is the adoption of lightweight materials, such as composites. Multifunctional composites are particularly interesting to reduce vehicle weight, for example by replacing components (e.g. embedding circuits to replace the use of wires) and improve efficiency, process and use conditions (e.g., health structural monitoring sensors into structural matrix). A breakthrough would be to have recyclable composites to replace existing plastic and (possibly) non-plastic components currently used. The Horizon Europe project REPOXYBLE (2023-2026, GA 101091891) is developing a new class of high-performance epoxy-biobased AdMs for the aeronautic and automotive sector, targeting safety, cost & energy effectiveness, recyclability, and thus sustainability. For this, a holistic and iterative approach incorporating the Safe and Sustainable by Design (SSbD) concept has been applied since the early stages of material design, to bring in-depth knowledge on the whole material life cycle and possible impacts of our product solutions, toward performance and sustainability optimization. REPOXYBLE is facing a combination of technical challenges, given the ambition to replace existing materials (epoxy resins for the automotive, titanium for aeronautic), without compromising performances. It will work to minimize the resource and energy demand during manufacturing, and to achieve high recyclability and re-use of the building blocks (monomers & hardeners) and additives via optimized chemical recycling. A Multi-Criteria Decision Analysis (MCDA), multidisciplinary approach is being used integrating qualitative to quantitative data related to six Lines of Evidence: Performance, Technological, Safety, Sustainability, Legal and Economic aspects. Raw materials selection and testing, safety assessment and preliminary LCA considerations run in parallel leading to the selection of materials to use for our composite solutions. REPOXYBLE took advantage of the multidisciplinary team of the project, involving partners from all over Europe covering the whole material value chain, and contribution from external experts. Current findings concerning methodologies, barriers and opportunities for practical implementation of SSbD, advancements in product solutions, will be presented.

#### **7.04.P-We568 Learning from Safe-by-Design for Safe-and-Sustainable-by-Design: Mapping the Current Landscape of Safe-by-Design Reviews, Case Studies, and Frameworks**

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With the introduction of the European Commission's "Safe and Sustainable-by-Design" (SSbD) framework, the interest in understanding the implications of safety and sustainability assessments of chemicals, materials, and processes at early-innovation stages has skyrocketed. Our study focuses on the "Safe-by-Design" (SbD) approach from the nanomaterials sector, which predates the SSbD framework.

In this assessment, SbD studies have been compiled and categorized into reviews, case studies, and frameworks. Reviews of SbD tools have been further classified as quantitative, qualitative, or toolboxes and repositories. We assessed the SbD case studies and classified them into three categories: safe(r)-by-modeling, safe(r)-by-selection, or safe(r)-by-redesign. This classification enabled us to understand past SbD work and subsequently use it to define future SSbD work so as to avoid confusion and possibilities of “SSbD-washing” (similar to greenwashing). Finally, the preexisting SbD frameworks have been studied and contextualized against the SSbD framework.

Several key recommendations for SSbD based on our analysis can be made. Knowledge gained from existing approaches such as SbD, green and sustainable chemistry, and benign-by-design approaches needs to be preserved and effectively transferred to SSbD. Better incorporation of chemical and material functionality into the SSbD framework is required. The concept of lifecycle thinking and the stage-gate innovation model need to be reconciled for SSbD. The development of high-throughput screening models is critical for the operationalization of SSbD. We conclude that the rapid pace of both SbD and SSbD development necessitates a regular mapping of the newly published literature that is relevant to this field.

#### **7.04.P-We569 Safe and Sustainable Innovation Approach: Towards an agile system for dealing with innovation**

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Advanced materials offer society economic and technological opportunities, but technological innovations pose a challenge to governance of human and environmental safety as well as to governance of sustainability due to the large difference in the pace between innovation and the development of suited governance. To cope with this difference in pace and move towards safe and sustainable innovations, an agile and robust system is needed that is pro-active and interactive, that deals with the fast pace of knowledge generation and helps to ensure that knowledge is transferred to adaptable safety and sustainability regulation. In this sense, the Safe and Sustainable Innovation Approach (SSIA) aims at reducing the time gap between the emergence of technological innovations and the development of suitable safety and sustainability assessment tools and frameworks. SSIA combines:

- Safe-and-Sustainable-by-Design (SSbD) concept, which is a central element of the EC Chemicals Strategy for Sustainability, recommends innovators to integrate safety and sustainability considerations as early as possible into the innovation process; and
- Regulatory Preparedness (RP) which aims to improve the awareness and anticipation of regulators to facilitate the development of adaptable (safety and sustainability) regulation that can keep up with the pace of knowledge generation and innovation of nanomaterials, nano-enabled products, and advanced materials.

Both the SSbD and RP concepts are supported by a process to share and exchange knowledge, information and views in a Trusted Environment (TE). SSIA thus relies on dialogue between innovators and regulators. SSIA brings significant cultural changes in comparison to current innovation management:

- SSbD incorporates considerations as early as possible in the innovation process thus the scope shifts from remediation (or precaution) to prevention.
- SSIA considers any concern on safety and sustainability, which may be generated by the innovation, from the design phase to the entire life cycle of any materials or articles obtained by this innovation.

The growing development, complexity, and opportunities brought by nano- and other advanced materials, are used as a case study for the development of the tools that should enable the implementation of SSIA. Work performed in the OECD Working Party for Manufactured Nanomaterials (WPMN) SSIA Steering Group will be presented.

#### **7.04.P-We570 Sustainable product innovations for fast-moving consumer goods – Learning from companies’ experiences for a more effective and accessible ‘Safe and Sustainable by Design’ framework**

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Advanced materials (including complex chemicals) represent a major opportunity for disruptive innovation as they can unlock novel benefits for consumers and manufacturers. In that context, it is essential to ensure that the new products and innovations related to these materials are safe and sustainable by design. The European Commission Joint Research Centre (EC JRC) has proposed the ‘Safe and Sustainable by Design’ (SSbD) assessment framework as an external, policy-supported methodology for sustainable product innovation. The framework is intended to support companies in their innovation journey by providing them with appropriate tools and methods.

Unilever is a global fast-moving consumer goods company with considerable portfolio diversity. Safety and sustainability are deeply embedded in our innovation process; a team of internal experts work closely with R&D teams from the earliest stage of

product development. Hence, we support the ambition of the SSbD framework to integrate safety and sustainability in the core of innovation and help drive competitive and sustainable growth in the European Union.

Drawing on our downstream user point of view and our experience in designing safe and sustainable products for decades using our internal approaches, we offer some reflections on the SSbD framework proposed by the EC JRC. We have produced a case study in which we first describe our current approach to safety and sustainability during innovation. Our case study focuses on a biosurfactant used in Home Care products. Insights drawn from the case study are synthesised to offer recommendations for further development of the JRC SSbD framework. We focus our recommendations on methodological and technical aspects as well as practical feasibility of implementing the framework for companies of all sizes and with a range of in-house technical expertise in safety and sustainability.

#### **7.04.P-We571 Safe, Sustainable and Recyclable by-Design (SSRbD) integrated approach applied to polymeric systems**

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A shift in mindset is required to ensure that newly developed materials integrate functionality with safety and sustainability aspects from the innovation phase through to the development of the final product. The Safe, Sustainable and Recyclable by-Design (SSRbD) concept identifies safety and sustainability hotspots early in the innovation and product development process to minimize potential hazards and exposure, maximizing sustainability while maintaining product functionality.

The purpose of this work is to present an integrated SSRbD approach to ensure SSRbD material development. The proposed method will assist material designers to integrate safety and sustainability assessments into the design process and facilitate dialogue with assessment experts. The integrated approach is an iterative process that considers the technology readiness level (TRL), avoids a potential gate effect, integrates a life-cycle vision and covers the five steps of the assessment of the safe-and-sustainable-by-design (SSbD) framework proposed by the European Commission (commission recommendation (EU) 2022/2510). The operationalization of the SSRbD integrated approach for polymeric materials is tested on three cases studies in the building, transport and the packaging sectors. The first step conducted early in the innovation process is to obtain an overview of each case study by filling a SSRbD matrix including safety and the three pillars of sustainability (environmental, social and economic) mapped across all life cycle stages. Based on the analysis of the SSRbD matrix, a second iteration loop is carried out at the middle stage of the innovation process.

The challenge is to establish the life cycle and development diagram (LCDD). The next steps will involve merging three life cycle inventories (hazard, exposure, LCA/LCC) into one to improve assessments efficiency. This integrated approach, developed and tested for plastics, could contribute to the operationalization of the SSbD framework and can easily be applied to other sectors.

#### **7.04.P-We572 Safe and Sustainable by Design Framework for Advanced Nanomaterials: The Harmless Approach**

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The establishment of a European Framework for 'Safe and Sustainable by Design' (SSbD) for chemicals and materials is an important step towards the production of safer and more sustainable products. Yet, it requires resources that are not compatible with the expected commercial value at early innovation stages and makes use of methods that are not fully applicable to emerging materials such as advanced materials (AdMa). The purpose of the HARMLESS approach is to build a framework that considers variability in data availability and resources along the innovation process, is applicable to nano-enabled AdMa and is cost-effective. This framework is aligned with the EU framework, complemented with a flexible stage-gate model and implements New Approach Methodologies (NAMs) tailored to nano-enabled AdMa SSbD assessment, for both innovators and regulators.

Making sure there was mutual understanding between safety and sustainability communities, knowledge and know-how were gathered from experts within the HARMLESS consortium to create a knowledge-exchange platform leading to a blueprint of the HARMLESS framework. This was tested on industrial case studies to align with industry.

The HARMLESS framework includes three innovation stages: ideation, lab scale and pilot scale. It starts with the categorization module of AMEA (Advanced Material Earliest Assessment) at the first innovation stage, testing the applicability of the framework to the business case. At each innovation stage, five different modules enable the assessment of 1) intrinsic safety, 2) occupational and environmental safety at production, manufacturing and end-of-life, 3) consumer and environmental safety at use, 4) environmental sustainability at production and manufacturing and 5) environmental sustainability at use and end-of-life. Within each module, design principles are given to guide the user in making products as safe and sustainable as possible, and methods and tools are suggested to facilitate the SSbD assessment. NAMs are prioritized to make SSbD assessment as cost-effective as possible. After all modules are completed, a gate enables the user to balance the safety and sustainability of their product with cost and performance, thereby assessing the relevance of going to the next

innovation stage. This framework guides the creation of an online decision support system, which will be publicly available to help industry making safer and more sustainable AdMa-containing nano-enabled products.

#### **7.04.P-We573 A science-based innovative dashboard tool to operationalise Safe-and-Sustainable-by-Design**

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The “Safe-and-sustainable-by-design” concept is one of the key innovations in the European Green Deal, representing a fully pro-active stance that is taken to phase out harmful chemicals and ensure that new chemicals are designed to serve desired societal functions with no or minimal harm. One of the biggest challenges in applying SSbD in practice is the lack of data early in the innovation process, because novel compounds are typically characterized by absence of data. How then to operationalize a good idea (SSbD) with (almost) no data?

This presentation is based on a forward-looking vision on how an ideal and operational environmental safety assessment for data-poor/lacking substances can be performed. A dashboard was envisaged, in which companies can enter simple information, such as the CAS-number of a chemical, to yield information on (un)safety in the format of a meaningful heatmap. The dashboard interface (being use-oriented also for Small and Medium Enterprises) is based on the innovative use of available ecotoxicity data. The scientific basis for bridging data gaps in ecotoxicity data was adopted from Viljanen et al. (2023) SAR QSAR Env Res 34 (765–788).

In the presentation, the dashboard concept, its utility and its scientific underpinning are shown for a sample of ecotoxicity data. That is, a sample of ecotoxicity tests data taken from a curated database of >250k aquatic ecotoxicity data (underlying Posthuma et al., 2019, ET&C) was used to predict all missing ecotoxicity data for the full matrix of species\*compound pairs. The thus-completed species\*chemical matrix was sorted to yield an ecotoxicity heat map. The heat map represents the full array of empirical “all-species” Species Sensitivity Distributions, which can in turn be used for hotspot identification and SSbD explorations.

If so desired, users can see whether and in how far a novel molecule, designed for a function based on CAS-number, may pose harm in comparison to others. Outputs can be comparatively used to head for a “green portfolio” of company products, derive chemical footprints of products and much more. The prototype dashboard is being further developed, as the underlying ways for the development of practical and easy-to-use tools to bridge the data gap can serve multiple important purposes in SSbD application, environmental protection, assessment and management.

#### **7.04.P-We574 Socio-Economic Life Cycle-based Framework for Safe and Sustainable by Design of Advanced Materials**

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This study describes an innovative approach to socio-economic assessment of (advanced) engineered nanomaterials and nano-enabled products (NEPs) to support safe-and-sustainable-by-design (SSbD) decision making by industries in the early stages of product development. This semi-quantitative methodology is developed based on a sound conceptual framework grounded in the combination of social life cycle analysis and multi-criteria decision analysis methods and supports decision making based upon socio-economic impacts assessed over the full life cycle of a product. To facilitate its application by industries, the methodology was developed as an excel-based tool and is currently being transferred to a web-based self-assessment tool. This easy-to-use, cost- and time-efficient tool can guide users through their SSbD decision making regarding newly developed nanomaterials and NEPs and can also be applied to re-evaluate existing materials and NEPs in order to improve their sustainability from a socio-economic perspective. The relatively low requirements of this tool regarding the level of efforts and expert knowledge needed for its application make it a good starting point for initial assessment to highlight socio-economic issues in the value chain. As a stepping stone for a more holistic assessment, the S-LCA self-assessment tool is now being integrated as a mid-level sustainability assessment in the H2020 SUNSHINE project e-infrastructure for SSbD of MCNMs. Currently, testing and refinement of the tool in real case studies is being conducted including but not limited to photocatalytic ZnO/Silica complexes used in scratch and abrasion-resistant coatings for the construction sector, core-shell silicon carbide (SiC)-titania (TiO<sub>2</sub>) anti-stick coatings for use in consumer products and graphene oxide-based materials for electrodes and energy storage (batteries).

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#### **7.04.P-We575 SSbD application: widening Step 1 to environmental, economic and social considerations for early stage decision making**

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The metallization of plastics' industry emerged 50 years ago combining the benefits of processing, lightweight and cost of plastics, together with the durability, tribological and anticorrosion properties of metals. However, the traditional processing to obtain the metalization of polymers involves both, highly toxic substances (Cr 6) and also critical raw materials (Paladium). The concern about these substances is leading the research towards greener alternatives for plastics metalization.

FreeMe Project supports the development of two novel technologies, not yet systematically tested from the safety and sustainability perspective. FreeMe aims to couple the research and development of these technologies with an iterative safety and sustainability. This approach will support the identification of hotspots and criticalities and elaborating recommendations for improvement before the processes evolves in the TRL, avoiding further costs and research efforts.

The application of the SSbD has proved to be a powerful tool to identify the most critical aspects of the novel technologies. The SSbD strategy has been temporalized for its application in this Project, advancing in paralel to the technical research. The first assessment (Step 1. Intrinsic properties) was carried out through the identification in the ECHA system of the compounds used in the novel and classical technologies. Step 1 can be addressed at very early stages of the development (TRL 2-3), providing guidance for the selection of the compounds.

Even though it is not foreseen in the SSbD framework, it was found useful to develop a sustainability pre-assessment. The environmental review was focused in the quantification of the impacts associated to the production of each of the substances under study, through literature and databases information (EcoInvent). Additionally, a literature review of the traditional technology, establishing an environmental baseline.

From the economic perspective, the cost for the raw materials can be estimated from a literature and market, including environmental impact through a monetary valuation into the final cost. The materials and chemicals involved were also mapped to identify the main producers worldwide as well as the value chains and global trends associated to their production and commercialization. This will support economic and social decision making at a very early stage incorporating the sustainability dimension at low TRLs.

#### **7.04.P-We576 Ensuring regulatory Alignment in the Research & Innovation of five market-relevant Advanced Materials**

*Steffi Friedrichs, AcumenIST*

The MACRAMÉ Project on 'Advanced Characterisation Methodologies to assess and predict the Health and Environmental Risks of Advanced Materials' is fully aligned with the EU ambitions to secure the safety and sustainability of new chemicals, materials, products and processes in order to strive for zero pollution and toxic-free environments, as addressed in the EU's Chemical Strategy for Sustainability (2020), and in the European Green Deal (2019 & 2021); in doing so, the Project concentrates on methodologies that are applicable to nanomaterials, and widens them to 'Advanced Materials' (AdMas) – a material category that includes but surpasses that of 'nanomaterials' (EU, 'Definition of a Nanomaterial') - in commercialised products and that are aligned with the future-oriented innovation, safety and sustainability considerations of the OECD (OECD (2020)), the EU (EU (2022)), and several of its Member States (e.g. Germany (2021)). This will be achieved through development and demonstration of novel methodologies, and by advancing their harmonisation & standardisation on three MACRAMÉ Material Families of inhalable carbon-based AdMas of various morphologies and dimensions (Tiwari *et al.* (2012)), beyond spherical particles: (a) graphene-related material (GRM), (b) carbon nanofibres (CNFs), e.g., carbon nanotubes (CNTs), and (c) Poly Lactic-co-Glycolic Acid (nano)particles (PLGA). The focus on carbon-based AdMas addresses unsolved detection and characterisation issues, especially in complex media. In doing so, MACRAMÉ builds on >15 years of research and innovation (R&I) and knowledge pooling in nanosafety, formed through numerous European and international collaborations. MACRAMÉ will add value to the results of collaborations, such as the Malta-Initiative, and the Graphene Flagship Validation Service and Standardisation Committee, to proactively support EU industries in becoming world-leaders in clean technologies and products and achieving the Green Deal's ambitious timeline.

To ensure the maximum impact and engagement with industrial actors, policy-informing and –making, as well as standardising bodies, two MACRAMÉ Regulatory Risk Assessors Summits will be to enhance the chances of acceptance and advancement of the developed methods toward standardisation and harmonisation.

The discussions of the 1<sup>st</sup> Summit will be carefully reviewed and its results will be both published as part of a Project deliverable report and presented at the SETAC Europe 34<sup>th</sup> Meeting.

#### **7.04.P-We577 Enhancing the European Commission's Safe and Sustainable by Design Framework for Chemicals and Materials**

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Sala<sup>2</sup>, (1)European Commission - Joint Research Centre (JRC), Belgium, (2)Sustainable Resources Directorate, European Commission - Joint Research Center (JRC), Italy, (3)European Commission - Joint Research Center (JRC), Italy  
Key for the Commission's vision for achieving the green industrial transition is the safe and sustainable by design (SSbD) framework developed by the JRC. It proposes a methodology that enables the evaluation of the safety and sustainability aspects of chemicals, materials and products (SSbD) before they are placed on the market, and can as well be applied to goods already on the market. The framework integrates safety and sustainability dimensions for the first time. In addition, it enables safety and sustainability considerations throughout the entire life cycle of chemicals, materials and products, to be assessed from the beginning of the R&D until end-of-life, which is a shift in the current practice. The framework can also be used as a tool for comparative assessment, which will be key to replace the most harmful chemicals and materials with more desirable alternatives and this way support substitution.

In order to improve the relevance, reliability and operability of the SSbD framework in R&I activities, the European Commission (EC) published in December 2022 a Recommendation establishing a European assessment framework for safe and sustainable by design (SSbD) chemicals and materials. The Recommendation establishes a two-year testing period in which stakeholders are invited to test the framework and provide feedback.

The aim of the presentation will be to raise awareness and promote the Safe and Sustainable by Design concept, to present the results of the first testing period and to inform on the on-going and future activities.

**7.04.P-We578 Testing Tools for Suitability for SSbD in Early Phases of Innovation, Applied to BPA and Alternatives**  
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Within the PARC project (<https://www.eu-parc.eu/>), gathering a large number of research organisations and public bodies, one Task deals with Safe and Sustainable by Design for chemicals and materials, SSbD, in relation to the framework described by the European Commission in a recent study [1]. Experience of applying the suggested SSbD framework is so far limited. Various EU projects (e.g. PARC, IRISS) and national initiatives (e.g. Mistra SafeChem in Sweden) are in the process of advancing and/or evaluating tools for SSbD for a range of use cases. The authors are co-leading the work within PARC to test tools in case studies for SSbD and reports here on advancements in a case study on Bisphenol-A and selected alternatives.

In addition to Bisphenol-A, one similar compound, Bisphenol AP; and a significantly different one, Iso-sorbide, were chosen as test substances. Two rather broadly formulated application areas were also chosen for the further work, substituting BPA in polycarbonate food-contact bottles, and substituting BPA in an epoxy coating.

The purpose of the testing was not to find an optimum substitute, but rather to understand what different tools may provide in relation to the chemicals, applications, and given limited information.

The tool testing procedure called for volunteering experts to run one or several tools among the previously suggested ones, in the appropriate step of the SSbD framework: 1. Hazard assessment, 2. Exposure assessment, 3. Use scenario environmental and health risk, 4. Life cycle environmental sustainability and 5. Socioeconomic sustainability.

An important first part of the tool testing procedure was to provide only the structure in one or the other form, and the two selected intended application areas, in order to simulate an exploratory innovation stage.

initial findings:

- The complexity of SSbD lies in the breadth of assessment types that is required to cover the whole framework
- Safety and sustainability aspects have traditionally been dealt with separately; experts in the two domains have different backgrounds, training, and use of words. A lot of effort is needed to create a common understanding of SSbD.
- In early phases of innovation qualitative expert judgement, semi-quantitative models, and computational models, all have a role to play. Interestingly, this is a common denominator for all SSbD steps. And predictive models are needed, and are also available, for all steps.

**7.04.P-We579 Safe and Sustainable by Design strategies validated for toxicity reduction applied to active nanomaterials with antibacterial and antiviral properties**

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The development of coatings with antiviral and antimicrobial properties based on active nanomaterials (ANM), such as bio-based active materials and inorganic nanoparticles, are a promising alternative for minimise the risk of spread infections from harmful pathogens in different high traffic objects surfaces (metal, plastic and textiles).

However, these developments could have an impact on environmental and human health if the substances used for the coatings developments are not safe or have bad performance in terms of durability or efficiency. Therefore, the implementation of safe- and sustainable-by-Design (SSbD) approaches is a key element to guarantee their harmlessness. Developing ANM safely-tailored for the final applications when they will be used in coatings, with reduced cytotoxic and ecotoxic effects, has been one of the main goals of the HE SUSAAAN Project.

The antibacterial power of zinc oxide nanoparticles is well known, but they also have effects at the cellular level and on aquatic organisms. One of the strategies applied to some of the ANMs developed in the project has been the encapsulation of these nanoparticles in silica, in order to minimise their hazards. To validate the reduction, *in vitro* toxicological studies have been performed on human cell lines representative of the main entry routes into the organism (inhalation, dermal, oral) to evaluate cytotoxic and dermal irritation effects, as well as bioassays using aquatic organisms according to OECD procedures (201, Algae growth inhibition test; and 202, *Daphnia sp.*, acute immobilization test).

The results have shown a significant reduction of the toxic effect of ZnO nanoparticles encapsulated in silicon oxide on cell viability in the case of the three lines used (HaCaT, A549 and CaCo-2), as well as in aquatic organisms.

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#### **7.04.P-We580 Challenges in the Evaluation of the Environmental Sustainability of Advanced Materials: the Case of Printable Electronics**

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Printed electronics (PE) have emerged as a possible approach for realizing cost-effective wearable and conformable devices. Over the course of the last two decades, organic semiconductors have been employed in a plethora of applications promising to revolutionize the market for everyday-life electronic circuits and sensors. Organic semiconductors are particularly interesting due to their compatibility with solution process deposition techniques compatible with industrial scale production (such as screen printing, roll-to-roll process and inkjet or 3D printing). Our research uses these fabrication processes to realize devices on glass, plastic and textile substrates for application in gas sensing, physiological parameter, analytes and radiation detection. Even though PE is a potential great breakthrough for an environmentally safe electronic production, researchers of the field are still struggling to ensure their compliance with Europe's Green Deal goals. Some challenges are common for all LCAs involving innovative processes, like the foreground data collected at lab-scale, which frequently don't match those of the operating production facilities. The required upscaling procedures also bring along a higher level of uncertainty within the impact assessment. Moreover, a new set of challenges are likely to be encountered when working with advanced materials (AdMa). To ensure a safe and sustainable by design approach, early-stage environmental evaluations must be performed. This often leads to the impossibility of carrying out a cradle-to-grave LCA because AdMa applications are still unclear. Therefore, system boundaries are narrowed down, and the functional unit refers to the minimum viable product, thus excluding other non-essential standard components and custom design parts. Also, the Life Cycle Inventory poses challenges, as the manufacturing process of AdMa often rely on uncommon substances for which datasets are not available, nor there is an urge to create them, since they would only be used by a niche of analysts. This situation is frequently coupled with limited literature and with data that cannot be early-published, due to the secrecy and non-disclosure agreements related to intellectual properties rights and patents. Therefore, to further the studies on AdMa, these issues need to be addressed, as both researchers and industrial partners would find value in building a network of shared data, methodology and knowledge.

#### **7.04.P-We581 Assessing the Impact of Nanomaterials on *D. magna*, *R. subcapitata*, and *E. foetida*: Towards Safer and Greener Nanomaterials through SSbD strategies - Titanium Dioxide Nanoparticles as a case study**

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Nanomaterials, with their unique physicochemical properties, are promising for industrial applications such as electronics, medicine, and consumer products. However, their potential impact on human health and the environment is concerning. As nanomaterials enter the environment, they can interact with biological systems, causing adverse effects on organisms and ecosystems. Toxicity and ecotoxicity of these nanomaterials, rely mainly on their physicochemical properties, such as size, shape, surface charge, and solubility.



Release of nanomaterials into the environment, poses threats to ecosystems, raising questions about their long-term ecological consequences. To address these challenges, Safe- and Sustainable-by-Design principles are being developed and applied to engineer nanomaterials to reduce their toxicity and its environmental impact, and promotes the use of green synthesis methods, optimizing energy and resource use and design materials with minimal ecological impact.

Key SSbD strategies include (1) Hazard assessment and identification of nanomaterials with potential hazardous effects (2) Modifying the physicochemical properties of nanomaterials to reduce their toxicity and ecotoxicity, (3) Applying environmentally friendly methods for the synthesis and manufacturing of nanomaterials (4) Designing nanomaterials to reduce their environmental persistence and promote biodegradation, (5) Life cycle assessment, assessing the environmental and health impacts of nanomaterials throughout their entire lifecycle.

In the context of SbD4Nano and DIAGONAL projects ecotoxicological studies have been carried out, monitoring the effect of nanoparticles on different organisms, such as *Daphnia magna*, *Raphidocelis subcapitata*, and *Eisenia foetida*. This ecotoxicity assessment has not only been carried out to assess the effects of nanomaterials on these organisms but has also played a key role in the modification of nanomaterials following SSbD strategies to obtain safer and more sustainable nanomaterials with a reduced impact on health and the environment.

The ecotoxicity tests carried out should always be standardised, so that the tests are done in accordance with what is agreed by different organisations providing reliable results. The test on *Raphidocelis subcapitata* was carried out according to OECD 201, the test on *Daphnia magna* according to OECD 202 and the test on *Eisenia foetida* according to OECD 207.

#### **7.04.P-We582 Safe and Sustainable by Design: Case Study of Microreactors and Bionanocompounds for Wastewater Treatment**

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Microfluidics, situated at the convergence of science and technology, is an evolving field dedicated to crafting small-scale devices for manipulating fluids and objects to achieve various functionalities, including environmental and biomedical analyses [1]. It showcases the potential of microreactors and bionanocompounds as a viable solution for remediating water sources contaminated by dyes from the textile industry. To ascertain their suitability, life cycle assessment (LCA) and operational efficiency analysis have been conducted [2]. This study reveals a promising pathway for designing innovative wastewater treatment systems that integrate high-performance microreactors and bionanocompounds. Moreover, the evaluation of potential toxicity risks of these nanoparticles have been conducted using a zebrafish animal model [3]

In alignment with the "Safe-and-Sustainable-by-Design" (SSbD) approach, this study proposes for integrating safety and sustainability aspects into the application of bionanocompounds for wastewater treatment. The study aims to identify and address industrial and regulatory challenges associated with this process. Following the guidance and initiatives of the European Commission, this study seeks to align its recommendations with broader regulatory frameworks, thereby promoting the responsible and sustainable use of innovative technologies.

Furthermore, this study delves into the application of the SSbD framework, emphasizing its potential impact on the design and implementation of wastewater treatment systems. By incorporating SSbD principles, the study envisions a comprehensive and proactive approach to the development of bionanocompounds, ensuring their safety, sustainability, and efficacy. Ultimately, the research aims to contribute valuable insights and recommendations to guide future endeavors in achieving a safer and more sustainable application of bionanocompounds in wastewater treatment processes.

(1) S. Ortegón, P. A. Peñaranda, C. F. Rodríguez, M. J. Noguera, S. L. Florez, J. C. Cruz, R. E. Rivas, J. F. Osmá, *Molecules* 2022, 27, 6198. DOI 10.3390/molecules27196198.

(2) O. P. Fuentes, M. J. Noguera, P. A. Peñaranda, S. L. Flores, J. C. Cruz, J. F. Osmá, *Advances in Microfluidics and Nanofluids* 2021. DOI 10.5772/intechopen.96822.

(3) A. Guillén, Y. Ardila, M. J. Noguera, A. L. Campaña, M. Bejarano, V. Akle, J. F. Osmá, *Nanomaterials* 2022, 12, 489. DOI 10.3390/nano12030489

#### **7.04.P-We583 Investigation of ecotoxicological effects of fibrous and platelet-shaped advanced materials for deriving adapted testing strategies - the project FaPlaN**

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Advanced materials are a very heterogeneous group of materials, and used in many applications. Responsible use of these materials also includes the identification of potential threats to people and the environment as quickly and as precisely as possible. Hence it is unclear to date, whether OECD test methods in the field of ecotoxicology (already established and adapted for testing nanomaterials) may also be applicable for this class of materials.

Due to their special properties, fibrous and platelet-shaped materials represent advanced materials for which a reliable assessment of a potential environmental hazard based on the available test methods is challenging. The project “FaPlaN” presented here therefore aims to obtain information on the mechanisms of action (MoA) and thus a mechanistic understanding of potential toxic effects exerted by fibrous and platelet-shaped advanced materials. Based on this, methods will be adapted which will allow to identify related effects. By this, ecotoxicological assessment will be facilitated in the long term.

This poster will present results from a literature review on the effects of fibrous and platelet-shaped advanced materials in aquatic organisms, with a focus on identifying MoA. In addition, an initial proposal for test systems that will potentially be part of the future test strategy will be presented.

However, the main aim of this poster is to exchange experiences with other (eco)toxicologists to learn more about possible mechanisms of action and to take these into account when adapting the testing strategies as the project progresses. There are three key questions, which are of main interest.

Key question 1: Which physical-chemical material properties must be considered?

Key question 2: Which MoA are relevant for fibrous and platelet-shaped advanced materials?

Key question 3: How need test methods/test strategies be adapted for fibrous and platelet-shaped advanced materials?

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#### **7.04.P-We584 Eco/Genotoxicity Assessment Of Yeast-based Natural Astaxanthin Obtained Using Bio-Based Solvents**

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It is already known about the widespread use of synthetic pigments in various industrial segments, and their potential to cause adverse effects on human and environmental health. The development of natural pigments, such as carotenoids, within the principles of green chemistry has been explored. Among them, it stands out that processes should be designed to use and generate substances that possess little or no toxicity to human health and the environment. Thus, our objective was to evaluate the aquatic toxicity and mutagenicity of microbial carotenoids (i.e., astaxanthin-rich extract) obtained from the wet biomass of the yeast *Phaffia rhodozyma* in a biorefinery approach, using bio-based solvents for solid-liquid extraction. Aquatic toxicity was evaluated using acute tests with the freshwater microcrustacean *Daphnia similis*, the marine amphipod *Parhyale hawaiiensis*, and the fish embryo test (FET) with the zebrafish (*Danio rerio*). Mutagenicity was evaluated using an *in vitro* bacterial reverse mutation assay, the *Salmonella* microsome test, applying a battery of 5 strains (TA97a, TA98, TA100, TA102, and TA1535 of *Salmonella enterica* serovar Typhimurium) with the presence and absence of exogenous metabolic activation (+/-S9 mix). For aquatic toxicity tests, the data were modeled by a generalized logistic model. The data from the mutagenicity were analyzed using ANOVA/Tukey's test followed by a linear regression using the Bernstein model. The astaxanthin-rich extract presented acute toxicity to *D. similis* with a 50% effect concentration (EC50) of 5.7 mg/L (1.9-18.0). The extract did not present acute toxicity to *P. hawaiiensis*. In the FET test, the extract induced only low toxicity in all tested concentrations ( $\geq 10\%$  mortality), and at the end of exposure, the larvae that survived did not present any sublethal effects. The astaxanthin-rich extract was not mutagenic in the strains of *Salmonella* TA97a, TA98, TA100, TA1535 +/-S9, and TA102 +S9. For the strain TA102 -S9, the extract showed toxicity and was re-tested in non-toxic concentrations, and a negative response was obtained. The astaxanthin-rich extract has low toxicity for both *Daphnia* and fish and was not toxic for *P. hawaiiensis*; it also was not mutagenic under the tested conditions, revealing a good option for application with lower impact. To better understand the impact of the entire production process, toxicity and mutagenicity tests will also be performed with the bio-based solvents used.

#### **7.04.P-We585 Engaging chemists in ethical deliberations of relevance for chemical management**

Dasha Kaluyk, **Gunilla Oberg**, Emma Davy and Oksana Nadybska, University of British Columbia, Canada

Chemistry plays a central role in virtually all societal areas such as health, food, energy, housing, transportation, clothing and hygiene. It is therefore not surprising that the chemical industry is one of the fastest growing sectors in society. While bringing many benefits, the chemical industry also brings harms to humans and the environment. This means that any potential benefits of chemical innovations must be weighed against the potential harm. With the increasing importance and impact of the chemical sector, it is crucial that chemists engage in ethical deliberations of relevance for chemical management and use their expertise to contribute to wise decisions. Such deliberations are complicated not only because it is challenging to assess the potential harm of chemicals but also because the ‘goods’ and the ‘bads’ are unequally distributed in society, with the most vulnerable populations commonly more exposed to hazards than other groups.

Some argue that chemists, with their specialist knowledge, have a moral obligation to engage in science-based considerations, particularly those that involve trade-offs. To be able to contribute constructively, chemists need to have a basic understanding of what it means to engage professionally in ethical deliberations of relevance for chemical management. Chemistry education

does, however, rarely include learning how to identify and weigh aspects of relevance for different interest holders such as the industry, governments, consumers, disproportionately exposed groups, and the general public.

This presentation analyzes ethical guidelines for chemists in light of research on experts' moral obligations and discuss in what ways a strengthening of ethical standards in the field of chemistry might benefit the sector and society. It describes a recently launched seminar-based undergraduate honors class where students learn to identify ethical dilemmas and develop alternative action plans, using a case-study approach.

## Track 8. Special Sessions

### 8.01 A Snapshot on Weapons and Military Chemicals in the Environment - Present Issues and Legacies from the Past

#### 8.01.T-01 The impact of Chemical Warfare Agents in the Environment

*Miguel A. Sierra, Universidad Complutense de Madrid, Spain*

To understand the impact of chemical warfare agents (CWA) in the environment, it is necessary first to understand what a CWA is. According to the Organization for the Prohibition of Chemical Weapons (OPCW), integrating the Chemical Weapons Convention (CWC), chemical warfare agents (CWAs) are chemicals used to cause intentional death or harm through their toxic properties. Several incorrectly denominated chemical warfare agents are not included within this definition. Herbicides like 2,4-D and 2,4,5-T (orange agent), dioxines, white phosphorous and many other chemicals that have been used in war, having serious environmental impact fall outside this definition.

This presentation will focus on the impact of OPCW Schedule I chemicals in the environment.[1] Lessons learned from the massive use of CWA in the WWI and the Iran-Iraq war pointed to devastating effects in the environment (some remaining after more than 100 years of their use). Moreover, the enormous stockpiling of chemical weapons derived from the Cold War still has consequences. It should be noted that under the CWC premises, it is required not only the complete destruction of chemical weapons (CWs) but also mandates that environmental and public health be fully protected during the process of disarmament and disposal. The CWC specifically outlaws such disposal methods as open-pit burning, land burial, and sea-dumping. The procedures to destroy CWAs, rendering these chemical harmless to the environment, have been exquisitely tuned-up during the last 25 years, and they will be discussed. Despite the completely destruction of the declared CWs by the OPCW states parties, abandoned chemical weapons in projectiles and other dispersion media, together with uncontrolled dumping of munitions or raw CWA in several places around the world, have serious consequences for the environment and human beings. Approaches to solving this environmental problem will be presented.

[1] For the definition of Schedule 1 Chemicals see: <https://www.opcw.org/chemical-weapons-convention/annexes/annex-chemicals/annex-chemicals>.

#### 8.01.T-02 Training activities in military ranges in Italy: environmental and health protection issues

*Daniilo Coppe<sup>1</sup> and Manuele Bernabei<sup>2</sup>, (1)Istituto Ricerche Esplosivistiche, Italy, (2)Italian Air Force, Italy*

The training activities of the Italian Armed Forces are carried out in 86 military ranges distributed throughout the national territory. These activities, which produce potentially polluting emissions and residues for the environment and human health, are fundamental to ensure the country has a prepared and effective defense instrument.

The growing attention in society regarding environmental protection, and the health/safety of workers has characterized the Italian political scenario in the last 30 years. At the same time the operational training in military ranges of the Armed Forces has been even more necessary due to their commitment outside national borders.

The Armed Forces growing awareness to environmental issues in operational training activities results in environmental regulations for fire activities carried out within all of Italian military ranges, monitoring the environmental matrices with chemical assessments and with numerical models to predict the dispersion of the polluting substances produced and, finally, with holding training courses for environmental experts. These activities were conducted in cooperation with the national environmental agency (ISPRA), the national health institute (ISS) and the renewable energy agency (ENEA).

This work examines two case studies involving two military ranges present in Sardinia (Salto di Quirra and Capo Teulada) where several studies defined their chemical and geolithological characterization. The results highlight the exceeding of some concentration limit values (i.e. arsenic and antimony) were due to geolithological variations in the composition of the soil rather than to military activities. The presence of uranium depleted was never found, and the use of thorium-containing weapon systems did not entail any radiological relevance for the environment. It was also determined, through studies on the dispersion of pollutants, that discontinuous and infrequent emission produced by weapon systems and their rapid dilution in the atmosphere, never led to exceeding the limit values or the environmental criticalities which characterize, for example, urban centers with a high density of automotive circulation.

The military ranges in Italy were established between 1950 and 1980 and half of them (43 out of 86) are today overlapped or adjacent to protected areas defined by European directives for the protection of habitats and species, approved in Italy in the 2000s, 30 years after the establishment of those 43 military ranges.

Finally, based on the European database Natura 2000, it results that habitats and species in those areas have remained unchanged since 2012 until today. This demonstrates how the existence of military ranges since the middle of the last century constitutes a potential source of pollution but, in fact, still represents today an effective defense against anthropic settlements for the protection of habitats and species.

## 8.02 Are We There Yet? – Ways Forward for Mechanistic Effect Modeling in Environmental Risk Assessment

### 8.02.T-01 Industry opinion on model development and submission – Challenges and requests

*Nika Galic<sup>1</sup> and Thomas G Preuss<sup>2</sup>, (1)Syngenta AG, Switzerland, (2)Bayer AG - Crop Science Division, Germany*

Mechanistic models are a promising tool for the environmental risk assessment of plant protection products. This was also acknowledged by regulating authorities, such as EFSA, by providing guidance on how to develop and document models (EFSA Good Modeling Practice) and how to apply state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms (EFSA 2018) .

This talk will provide an overview of the challenges encountered by industry, here represented via CLE, during the development and submission of mechanistic effect models for environmental risk assessment. Why we have very good experience in using effect models internal to understand how active ingredient act on different organisms and to optimize future active ingredients already in early phases, we see a reluctancy of regulatory authorities to investigate the potential of this methodology to answer the risk assessment questions. We will address the complexities and hurdles encountered during the development phase, emphasizing the need for dialogues with regulatory authorities. One of the main disadvantage in the application of models for pesticide risk assessment is that it does not fit into specific boxes of the risk assessment. Currently regulatory risk assessment is based on biotest in which several endpoints are evaluated over time. From all this endpoints the worst-case endpoint at the end of the experiment is selected to be compared to an exposure estimate. All the discussions within a risk assessment is about which of the endpoints and which of the studies to select. However, very often effect models can interpret the differences between the studies (e.g. food, temperature, exposure situation) and can explain the whole dataset.

Furthermore, we will share our experiences with model submission, shedding light on the challenges faced and the constructive feedback we anticipate or have received. We will also outline the requests and expectations from regulatory authorities, highlighting the need for more comprehensive and constructive feedback to improve the evaluation process.

By providing an industry perspective on model development and submission, we aim to contribute to the discussion on refining risks to non-target organisms. Our insights will offer a comprehensive understanding of the challenges faced, the feedback anticipated, and the requests we have to improve the overall process.

### 8.02.T-02 Regulatory perspective on model evaluation – Challenges and feedback

*Matthias Fürst<sup>1</sup> and Rachel Sharp<sup>2</sup>, (1)Austrian Agency for Health and Food Safety (AGES), Austria, (2)European Food Safety Authority (EFSA), Italy*

Effect models have long been recognised as useful tools in ecological research, offering insights into the dynamics of ecosystems and their response to various stressors. With the publication of the “*EFSA Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products*” in 2014, clarity was provided on the expectations on how effect models should be tailored to meet the requirements of regulatory ecological risk assessment. The industry has since started to implement these models in dossiers for active substances and plant protection products, encouraging regulators to engage with effect models and to evaluate them in ecological risk assessments. This presentation will feature the perspective of regulatory authorities sharing their experiences, both positive and negative, in assessing these models. They will highlight the primary challenges encountered during the evaluation process and propose potential solutions to improve the overall evaluation procedure in future applications. These insights should serve as a basis for the more detailed discussions during the panel session.

### 8.02.T-03 CRO’s viewpoint on model development and evaluation – Challenges and feedback

*Oliver Jakoby<sup>1</sup> and Benoit Goussen<sup>2</sup>, (1)RIFCON GmbH, Germany, (2)ibacon GmbH, Germany*

The application of mechanistic effect models has gained increased attention in the higher-tier environmental risk assessments, particularly in refining the evaluation of risks to non-target organisms. These models and model applications undergo rigorous evaluation by regulatory authorities. Contract Research Organizations (CROs) occupy a distinct role within this field, actively participating in all steps, from model development to submission, communication, registration, and evaluation.

Our presentation will delve into the specific challenges encountered during the development of these models, illuminating the complexities involved, the required expertise and time demands. We will emphasize the need for fostering continuous communication and collaboration among stakeholders to ensure the suitability and acceptability of the models.

Furthermore, we will highlight the feedback inherent in the model submission and evaluation process, drawing insights from the experiences of CROs. Thereby, we aim to provide insights into the iterative refinement process essential for enhancing model robustness and regulatory compliance.

Finally, we will discuss options to improve the collaboration between CROs, academia, regulatory authorities and industry. By enhancing cooperation among all stakeholders, we expect a more streamlined and efficient model application and evaluation process, improving today’s and future environmental risk assessments.

## **8.02.T-04 Academic insights on model development and evaluation – Status and potential improvements**

*Bas Buddendorf<sup>1</sup> and Sandrine Charles, PhD, HDR<sup>2</sup>, (1)Environmental Risk Assessment, Wageningen University & Research (WUR), Netherlands, (2)Umr Cnrs 5558, University Claude Bernard Lyon 1, France*

Models are increasingly used to inform and support decision making in environmental risk assessment (ERA). These models are becoming increasingly complex and data-intensive, which presents a challenge for end users without a strong quantitative or modelling background. Therefore, model developers need to be made more aware of what constitutes good practice in model development, including appropriate documentation of models for users and the ability for users to critically review the usability of models. As academic researchers developing effect models, we feel the obligation to ensure that models of appropriate quality can be critically evaluated and eventually considered to be fit-for-purpose for the use in the risk assessment for chemicals. We will enumerate what we consider as the most critical points for developing parsimonious and operational models, rather than theoretical and deep mechanistic models, while still ensuring an appropriate complexity and quality of models. As well, from an academic point of view, it is required to maintain the possibility of investigating both methodological research questions and operational objectives, the latter feeding the former and vice versa. From a helicopter perspective, we will highlight our needs in model evaluation, emphasizing the need to be able to look under the hood of a model (e.g., domain of applicability), and identify some of the main challenges and issues for model development and evaluation. We will end our presentation with some feedback from our personal experience, before making some suggestions for the future.

## **8.03 Beyond the Conventional Ecotox Endpoints - Advances to Unravel Low, Chronic Exposure Risks**

### **8.03.T-01 How to implement non-conventional endpoints in experimental ecotoxicology**

*Nico van den Brink, Wageningen University & Research (WUR), Netherlands*

### **8.03.T-02 When are non-conventional endpoints relevant for population modelling and how to implement them in modelling**

*Nika Galic<sup>1</sup>, Thomas G Preuss<sup>2</sup> and Pernille Thorbek<sup>3</sup>, (1)Syngenta AG, Switzerland, (2)Bayer AG - Crop Science Division, Germany, (3)Apd/EE, BASF plc, United Kingdom*

### **8.03.T-03 Behavioral consequences in wildlife birds, through telomere shortening due to chronic exposure to urban pollution**

*Matteo Schiavinato<sup>1</sup>, Shivani Ronanki<sup>2</sup>, Ignacio Miro Estruch<sup>3</sup>, David Gómez Blanco<sup>4</sup>, David Spurgeon<sup>5</sup> and Nico van den Brink<sup>4</sup>, (1)University of Padova, Italy, (2)Department of Toxicology, Wageningen University & Research (WUR), Netherlands, (3)Toxicology, Wageningen University & Research (WUR), Netherlands, (4)Wageningen University & Research (WUR), Netherlands, (5)UK Centre for Ecology & Hydrology (UKCEH), United Kingdom*

According to literature data, urbanization may influence the expression of certain personality traits in free-living birds and alter their inter-individual variability in some behaviors. But how could live in the city influence animal behavior? Since the world population is growing exponentially, the associated urban pollution due to industrial releases or exhaust gases from traffic is rising. Exposure to such chemicals could lead to increased oxidative stress in animals, potentially disrupting the balance between oxidants and the antioxidant systems. Telomeres, non-coding DNA sequences located at the end of the linear chromosomes, are especially sensitive to oxidative damage because they especially target their guanine bases. These damages initiate the DNA base excision repair pathway by DNA glycosylase, an enzyme that catalyzes the removal of the damaged nucleotides and, thereby, parts of the telomere, leading to the shortening of the sequences. For this reason, the overall length of the telomere in bases, has been used as a proxy for individual or populations health status, since this shortening due to oxidative stress accelerates the normal telomere length reduction that happens due to aging, leading to premature biological senescence. Recent empirical evidences shows how individual differences in telomere length are also associated with differences in behavior: generally, the shorter the telomeres, the more impulsive and bold the birds are. The possible biochemical reason we are exploring that links telomere and behavior, could reside in the “telomere position effect” (TPE), a molecular mechanism that causes the reversible silencing of the genes at the end of the chromosome by the spreads of the telomeric heterochromatin. Therefore, the shorter a telomere becomes, the more the closer genes become expressed. TPE would provide a mechanism to incrementally alter the phenotype according to organisms' senescence through the different expression of specific genes involved in behavior, as the dopamine receptors. Chronic exposure to low levels of pollution, might increase oxidative stress and shorter telomere, influencing animals behavior and their future fitness.

### **8.03.T-04 Multigenerational long-term exposure to low concentrations of pharmaceuticals in an estuarine deposit-feeding polychaete**

*Martina Santobuono<sup>1</sup>, Mette Albreksen<sup>2</sup>, Wing Sze Chan<sup>1</sup>, Elettra D'Amico<sup>3</sup> and Henriette Selck<sup>1</sup>, (1)Roskilde University (RUC), Denmark, (2)Science and Environment, Roskilde University (RUC), Denmark, (3)INM, Roskilde University (RUC), Denmark*

Pharmaceuticals are a group of emerging contaminants that are increasingly present in the environment. Selective Serotonin Reuptake Inhibitors (SSRIs), such as the antidepressant sertraline, fall into the category of psychoactive drugs known for their reduced side effects. Consequently, in recent years, prescriptions of these antidepressants have increased.

Due to its lipophilic nature, sertraline can accumulate in sediments after being released into aquatic environments. Thus,

deposit feeders may be continuously exposed to lipophilic chemicals since they ingest sediment particles and feed on sediment organic matter. In marine ecosystems, deposit feeders, like the estuarine opportunistic benthic polychaete, *Capitella teleta*, play a key ecological role as decomposers, nutrient recyclers, pollutant biotransformers, and they further constitute a major food source for demersal fish.

Long-term exposures and exposures across multiple generations are some of the new frontiers in assessing the adverse effects of chemicals on non-target species. Despite this, there is limited knowledge of the sublethal effects of lipophilic chemicals on sediment-dwelling invertebrates, leaving the impacts on critical ecological benthic species largely unexplored.

Considering that exposure to antidepressants occurs long-term and at low doses, this study aimed to investigate whether environmentally realistic concentrations of the sediment-associated antidepressant sertraline impact the benthic deposit-feeder *Capitella teleta* across three generations. Both conventional (e.g., life-history traits) and non-conventional (e.g., egg allometry) endpoints were investigated to unveil sertraline's long-term effects on *C. teleta*.

### 8.03.T-05 Modelling pharmaceutical exposure and effects using a novel DEB organism

*Jacqueline Hilgendorf<sup>1</sup>, Neil Sherborne<sup>2</sup>, Diogo N. Cardoso<sup>3</sup>, Jesper Givskov Sørensen<sup>4</sup> and Susana Loureiro<sup>5</sup>, (1)CESAM - Centre for Environmental and Marine Studies & Department of Biology, University of Aveiro, Portugal, (2)Syngenta, United Kingdom, (3)CESAM - Centre for Environmental and Marine Studies and Department of Biology, University of Aveiro, Portugal, (4)Department of Biology, Aarhus University, Denmark, (5)CESAM-Centre for Environmental and Marine Studies and Department of Biology, University of Aveiro, Portugal*

Current environmental risk assessment (ERA) of chemicals is mainly based on acute toxicity data from high concentration exposures observing survival as a main endpoint. However, this approach differs significantly from the field, where organisms undergo long-term, low-level exposure, potentially leading to sublethal effects. The Dynamic Energy Budget (DEB) model can bridge the gap between ERA practices and realistic exposure scenarios, providing a framework for energy allocation throughout an organism's lifecycle. With DEBtox, the impact of chronic chemical exposure on sublethal endpoints can be modeled over time.

DEBtox models have received growing attention in recent years and are partly incorporated in ERA practices<sup>1</sup>. Although they are applied to various species, their use for sediment species like *Lumbriculus variegatus* is limited. Given *L. variegatus*' recommendation for sediment ERA and the advice to use the species for chemical testing more often in the future [2], understanding its individual-level responses to chronic exposure is crucial. However, its life history and chemical effects on the single life stages are less understood compared to most model species, and it has not been adapted to the DEB model yet.

In this study, a chronic experiment was conducted using the model chemical fluoxetine, an antidepressant well known for its environmental persistence and wide range of toxic effects. The three endpoints, survival, growth, and reproduction, were evaluated at seven time points (0- 28 days). Four fluoxetine concentrations (0, 0.0025, 0.25 to 25 mg/kg) were tested with three replicates for each time point. Data from the different time points was used to apply the DEB model to *L. variegatus*.

Constructing a DEB model for *L. variegatus* is especially challenging as the worms have a highly unusual reproduction mechanism involving fragmentation, which differs significantly from other DEB model organisms. We present how this reproduction can be mechanistically described within a DEB model and how this allows both increased and decreased fecundity to be quantified as a stress response.

1 Ockleford, C., et al., "Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms" *EFSA Journal*. 16.8 (2018).

2 EFSA, "Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface water" *EFSA Journal*. 13.7 (2015): 145 pp.

### 8.03.T-06 New Approach Methodologies for the identification of environmental hazard and risk drivers

*Jose Tarazona, Instituto de Salud Carlos III, Spain*

The current risk assessment paradigm, for both human health and the environment, was built in the 20<sup>th</sup> century based on apical endpoints typically measured in "observational" experimental studies. For the environment, endpoints connected to population effects, i.e. mortality, growth, and reproduction, were selected and included in the protocols for regulatory ecotoxicity testing. Toxicology has evolved and the current focus is to minimize animal testing and generate mechanistic information, facilitating a better understanding of the interactions of the assessed chemical with biological structures, mostly at the sub-organism level and emphasizing human relevance. NAMs providing mechanistic information can also support environmental assessments, but the use of these novel endpoints requires a paradigm shift. Current risk assessments focus on the identification of thresholds of "no-effect", such as the Predicted No Effect Concentration, and the assumption that, for example, the reproduction NOEC for a parthenogenic daphnia is also applicable, through an interspecies extrapolation factor, to other aquatic invertebrates with very different reproduction physiologies. NAM-based endpoints can be used to confirm or to challenge this assumption. With our increasing knowledge on wild species biology and ecology, information on effects observed at early events could be connected with possible consequences at individual level for different species in line with their specific biology, and to infer the consequences in terms of population dynamics. This presentation challenges the current focus on concentrations with "no-

observed effects” on traditional apical endpoints, and explores a new approach. The focus should be on the “observed” effects and their potential environmental impacts, through the identification of the drivers for hazard (vulnerable species and expected effects) and risk (potential for disturbing population dynamics and community structures). The proposed implementation is as hypothesis driven next generation environmental risk assessments (NGERA), combining exposure-driven hypothesis, based on emissions and environmental fate properties, with effect-driven hypothesis, based on NAM bioactivity and the understanding of the potential consequences leading the environmental impacts. The approach is flexible and applicable to generic regulatory assessments as well as to spatially and temporally explicit landscape-based ERA.

## **8.04 Empowering Sustainable Innovations: Leveraging Alternatives Assessment for Safe and Sustainable Design in Practice**

### **8.04.T-01 Methodological Needs and Opportunities between Alternatives Assessment and Safe and Sustainable by Design**

*Peter Fantke<sup>1</sup> and Kerstin von Borries<sup>2</sup>, (1)Quantitative Sustainability Assessment, Department of Environmental and Resource Engineering, Technical University of Denmark, Denmark, (2)Technical University of Denmark (DTU), Denmark*  
‘Alternatives Assessment’ (AA) and ‘Safe and Sustainable by Design’ (SSbD) are both emerging concepts that support the transition toward safer and more environmentally benign chemicals in consumer products and industry processes alike. While both concepts have overlapping elements with strong focus on chemical substitution for specific or across different applications, they are applied in different decision contexts and with that come with different methodological needs and boundary conditions. This talk will identify such needs and conditions for both concepts and discuss opportunities for synergies in scientific advances and application of these concepts in decision support.

### **8.04.T-02 Overview of Results of the SETAC High Level Round Table Sounding Board Consultations on Safe and Sustainable by Design**

*Annegaike Leopold, ibacon GmbH, Germany*

### **8.04.T-03 Collaborative Innovation for Sustainability: Steering Early-Stage Chemical Development with the Safe and Sustainable by Design Framework**

*Josse Hiram Moerman, Apeiron-Team, Belgium*

### **8.04.T-04 Government Efforts towards Safer Alternatives: Examples from Washington State**

*Saskia VanBergen<sup>1</sup> and Colleen McLoughlin<sup>2</sup>, (1)Washington State Department of Ecology, (2)SciVera LLC*

### **8.04.T-05 What is needed to Hasten Innovation towards Inherently Safe and Sustainable Chemicals, Materials, Products and Processes. NGO perspective**

*Tatiana Santos, European Environmental Bureau, Belgium*

## **8.05 Establishment of a Science-Policy Panel to Contribute Further to the Sound Management of Chemicals, Waste, and Pollution Prevention**

### **8.05.T-01 Background to the Open Ended Working Group and future science-policy panel - relevance of stakeholder involvement**

*Paige Charlotte Robinson, Natural England, United Kingdom*

### **8.05.T-02 Background to the European Horizon Scanning process, final themes, and questions**

*Paul van den Brink, Wageningen University & Research (WUR), Netherlands*

The United Nations’ Sustainable Development Goals have been established to end poverty, protect the planet, and ensure prosperity for all. Delivery of the Sustainable Development Goals will require a healthy and productive environment. An understanding of the impacts of chemicals which can negatively impact environmental health is therefore essential to the delivery of the Sustainable Development Goals. However, current research on and regulation of chemicals in the environment tend to take a simplistic view and do not account for the complexity of the real world, which inhibits the way we manage chemicals. There is therefore an urgent need for a step change in the way we study and communicate the impacts and control of chemicals in the natural environment. To do this requires the major research questions to be identified so that resources are focused on questions that really matter. In the paper by Van den Brink et al. (2018), we present the findings of a horizon-scanning exercise to identify research priorities of the European environmental science community around chemicals in the environment. Using the key questions approach, we identified 22 questions of priority. These questions covered overarching questions about which chemicals we should be most concerned about and where, impacts of global megatrends, protection



goals, and sustainability of chemicals; the development and parameterization of assessment and management frameworks; and mechanisms to maximize the impact of the research. The research questions identified provide a first-step in the path forward for the research, regulatory, and business communities to better assess and manage chemicals in the natural environment.

Reference: Van den Brink, P.J., A.B.A. Boxall, L. Maltby, B.W. Brooks, M.A. Rudd, T. Backhaus, D. Spurgeon, V. Verougstraete, C. Ajao, G.T. Ankley, S.E. Apitz, K. Arnold, T. Brodin, M. Cañedo-Argüelles, J. Chapman, J. Corrales, M-A. Coutellec, T.F. Fernandes, J. Fick, A.T. Ford, G. Giménez Papiol, K.J. Groh, T.H. Hutchinson, H. Kruger, J.V.K. Kukkonen, S. Loutseti, S. Marshall, D. Muir, M.E. Ortiz-Santaliestra, K.B. Paul, A. Rico, I. Rodea-Palomares, J. Römbke, T. Rydberg, H. Segner, M. Smit, C.A.M. van Gestel, M. Vighi, I. Werner, E.I. Zimmer and J. van Wensem (2018). Towards sustainable environmental quality: priority research questions for Europe. *Environ. Toxicol. Chem.* 37: 2281-2295

## 8.06 Regulatory Needs for Scientific Development

### 8.06.T-01 Bridging the gap between science and regulatory science in the EU: Regulatory perspective

*Wim De Coen, European Chemicals Agency (ECHA), Finland*

### 8.06.T-02 Bridging the gap between science and regulatory science in the EU: Academia perspective

*Christoph Schaefers, Ecotoxicology, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany*

### 8.06.T-03 Bridging the gap between science and regulatory science in the EU: Industry perspective

*Blanca Serrano, ECETOC, Belgium*

### 8.06.T-04 Panel discussion.

*Wim De Coen<sup>1</sup>, Blanca Serrano<sup>2</sup> and Christoph Schaefers<sup>3</sup>, (1)European Chemicals Agency (ECHA), Finland, (2)ECETOC, Belgium, (3)Ecotoxicology, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany*

Panel members:

Thomas Backhaus, RWTH Aachen, Germany  
Marie Collard, DSM-Firmenich, Belgium  
Miriam Diamond, University of Toronto, Canada  
Anu Kapanen, ECHA, Finland  
Joop de Knecht, RIVM, Netherlands  
Johannes Tolls, Henkel, Germany

Discussion topics:

#### 1. New scientific data vis-à-vis regulatory use

- How to improve uptake of new scientific approaches/information/data/ publications into the regulatory hazard and risk assessment?
- What are the biggest bottle necks?
- What is needed to make progress?

#### 2. Appeal of regulatory science

- Is regulatory science interesting enough for academia? If not, how to make it more attractive?

#### 3. Regulatory research needs

- ECHA has published Key Areas of Regulatory Challenges for the future:  
[https://echa.europa.eu/documents/10162/17228/key\\_areas\\_regulatory\\_challenge\\_en.pdf](https://echa.europa.eu/documents/10162/17228/key_areas_regulatory_challenge_en.pdf)
- Are regulators clear in problem formulation?
- What other emerging research topics would you consider relevant to the regulators?

#### 4. Improved interaction between regulators and academia

- How to improve the communication on regulatory scientific development needs between the regulatory bodies and academia?

- What is missing to close the gap between both worlds?

Set the context with predetermined questions

*Michelle Bloor, United Kingdom*

Question 1. How do we avoid the misuse of the phrase 'science-based'? What does it mean, is all data useful data, and what level of information is sufficient to make informed decisions?

Question 2. How do we avoid paralysis by analysis in decisions on chemicals? Are calls for more data slowing down the process? What data are truly needed to move forward, and what is needed by regulators?

Question 3. Multidisciplinary and transdisciplinary solutions are called for, but how do we (as SETAC members and stakeholders) facilitate and achieve effective collaborations between different disciplines involved in the design, production, and assessment of chemicals, on what topics, and to what end?

Question 4. What are the CSS's key science data gaps to both inform decisions on chemicals and shape future chemicals that are Safe and Sustainable by Design?

Panel discussion and interactive Q/A

*Michelle Bloor, University of Glasgow, United Kingdom*

- A moderated panel discussion will take place between seven invited speakers: Annegaike Leopold (Calidris environment, The Netherlands), Ksenia Groh (Eawag, Switzerland), Leo Posthuma (RIVM, The Netherlands), Bruno Campos (Unilever, UK), Paul Thomas (Kreatis, France), Hans Sanderson (Aarhus University, Denmark) and Christoph Schuer (Eawag, Switzerland). The discussion panel will address the questions selected by the audience. The audience will also join in the discussion.
- During and panel discussion, the audience will also be asked to complete several Mentimeter poll questions to stimulate further engagement (a QR code linking to a Mentimeter poll will be shared with the audience for responses).

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