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Review

Impact of per- and polyfluorinated alkyl substances (PFAS) on the marine environment: Raising awareness, challenges, legislation, and mitigation approaches under the One Health concept

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ABSTRACT

Per- and polyfluorinated alkyl substances (PFAS) have long been known for their detrimental effects on the ecosystems and living organisms; however the long-term impact on the marine environment is still insufficiently recognized. Based on PFAS persistence and bioaccumulation in the complex marine food network, adverse effects will be exacerbated by global processes such as climate change and synergies with other pollutants, like microplastics. The range of fluorochemicals currently included in the PFAS umbrella has significantly expanded due to the updated OECD definition, raising new concerns about their poorly understood dynamics and negative effects on the ocean wildlife and human health. Mitigation challenges and approaches, including biodegradation and currently studied materials for PFAS environmental removal are proposed here, highlighting the importance of ongoing monitoring and bridging research gaps. The PFAS EU regulations, good practices and legal frameworks are discussed, with emphasis on recommendations for improving marine ecosystem management.

1. Introduction

Per- and polyfluorinated alkyl substances (PFAS), colloquially known as "forever chemicals", have persisted in commercial production since the 1940s, mainly due to their water-resistant, stain-resistant, fire-resistant and anti-adherent properties. They are commonly used as stain repellents, nonstick cookware (Teflon®), food packaging, floor and ski wax, textile sealants (such as Gore-Tex®), firefighting foams, pesticides, pharmaceuticals, and building blocks in the chemical industry (Han et al., 2021). PFAS compounds can be found in many industrial facilities, commercial households and packaging products (DeLuca et al., 2021). However, considerable attention has been attracted by the alterations brought forward with the revised PFAS definition suggested by the Organization for Economic Co-operation and Development (OECD) (ENV/CBC/MONO(2021)25, 2021), which drastically broadened the range of fluorochemicals that are now part of the PFAS marketable universe, encompassing compounds with vastly different applications as well as with distinct ecological footprints (EF) and environmental dynamics.

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A synopsis of the PubMed entries reported over the last decade on PFAS (including perfluorooctanoic (PFOA) and perfluorooctanesulfonic acids (PFOS)) revealed an increasingly broader scope of research on this hot topic. As expected, most publications on PFAS (but also PFOA and PFOS) deal with exposure, toxicity, accumulation in various tissues (mainly protein-rich, such as liver and blood) and harmful effects on human, animal, and environmental health. However, a keyword search exposed areas with very few publications (studies and reviews), such as marine biodiversity and effects on marine organisms. The literature search results are shown in Supplementary information Figures FS1(A-D), in a graphical representation with the used keywords in the (x axis) and publications number in the (y axis) over the past one, five and ten years.

It is quite clear that PFAS pollution is becoming a growing global issue and reason for concern due to their environmental persistence, lenghty half-lives, bioaccumulative nature, toxicity and environmental mobility (Abunada et al., 2020). Numerous harmful impacts on marine ecosystems reflect on their inhabitants at all trophic levels, through bioaccumulation and biomagnification, which particularly affects apex predators (Stockin et al., 2021; Boisvert et al., 2019). Although adverse effects on animal (Panieri et al., 2022; Death et al., 2021) and human (Lohmann et al., 2020; Pelch et al., 2019; Sunderland et al., 2019) health have been well documented in numerous reports in the last decade, recent studies in animal models (especially mammals) may indicate multiple negative consequences for marine birds and mammals, including the distribution and abundance of their populations. PFAS influence on microbiodiversity has already been demonstrated in studies of the gut microbiota animals and humans (Beale et al., 2022a; Beale et al., 2022b; Chiu et al., 2020) or microbiota inhabiting contaminated sites rich in PFAS (Senevirathna et al., 2022). However, the long-term effects of PFAS will certainly affect genetic biodiversity (via mutations and recombination), species biodiversity, as well as ecosystem biodiversity, through habitat loss caused by increasing contamination. PFAS pollution threatens the role of the ocean as the cradle and support of all life on Earth, showing even increased toxicity in interaction with microplastics (MP) (Dai et al., 2022; Scott et al., 2021). Finally, a healthy ocean is crucial not only for the health of ecosystems and its dwellers, but also for climate balance and the global economy.

This comprehensive review provides an updated overview of legacy and newly defined PFAS on marine environment, focused on providing a clearer picture of their long-term and unpredictable effects on ocean ecosystems, wildlife and human health. It considers PFAS interactions with other emerging pollutants such as microplastics, climate change issues and bioremediation challenges. Also, this is one of the first reports that suggest mitigation approaches for the marine environment, as the literature on this is scarce or non-existent. Regulations and legal frameworks are still on its infancy and lack efficient implementation of effective actions to mitigate this emerging pollution problem. Therefore, future research and regulatory practices must concentrate on developing effective strategies to mitigate, even prevent, the harmfull effects of PFAS on the marine environment, as well as on ways to improve marine ecosystem management practices to cope with increasing pollution risks and with the rapidly growing PFAS market.

Finally, this overview includes a debating PFAS regulations and legal frameworks, highlighting recommendations for improvement of marine ecosystems management. It was outlined to reach a broad audience and multiactors in the fields of marine biology, microbiology, climate change, blue biotechnology, bioremediation, environmental pollution, and chemistry. We hope to inspire innovative research approaches to reduce PFAS pollution by raising awareness of the harmful effects of PFAS pollution in the context of the One Health[1] concept.

[1] https://www.who.int/health-topics/one-health#tab=tab_1

2. The revised definition leads to a wider range of PFAS

The definition of PFAS is at the epicenter of a productive discussion regarding which compounds should fall under its umbrella. The OECD has recently proposed a determination from the widely accepted conceptualization of PFAS as fluorine-saturated aliphatic carbon chains in favor of a more lenient, which embraces all chemical compounds with at least one perfluorinated moiety: "PFAS are defined as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), i.e., with a few noted exceptions, any chemical with at least a perfluorinated methyl group (—CF_3) or a perfluorinated methylene group (—CF_2—)" (ENV/CBC/MONO(2021)25, 2021).

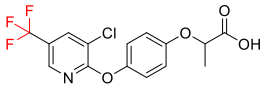
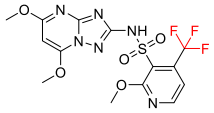
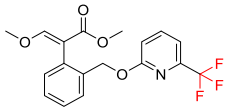
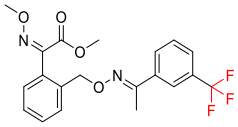
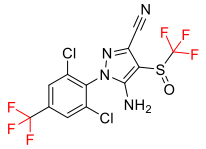
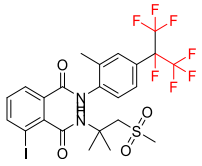
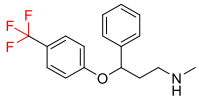
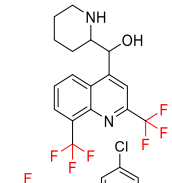
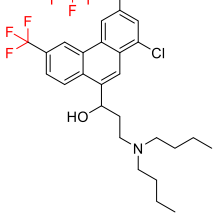
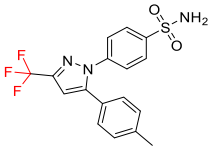
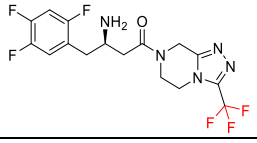
The impetus towards a revised PFAS definition aimed to rebut the narrow spectrum of the previously applied criteria while resolving many of its inconsistencies concerning the classification of other relevant perfluorinated hydrocarbons (e.g., perfluoroaromatic compounds) (Wang et al., 2021). Furthermore, the changing landscape of the fluoroorganics market as shown by the increasing number of polyfluorinated compounds being introduced in the pharmaceutical and agrochemical industry (Alexandrino et al., 2022; Han et al., 2021), warrants a clear and reproducible definition capable of better serving regulatory purposes (Wallington et al., 2021).

In its traditional connotation, PFAS refer to aliphatic perfluorinated molecules and mainly consists of perfluoroalkyl(ether) acids, fluoropolymers and perfluoropolyethers (Kwiatkowski et al., 2020). These PFAS have witnessed significant market traction for decades due to their unusual physical, thermal and chemical stabilities and water-repelling properties (Hamid et al., 2018). These favorable properties turned them into suitable templates for developing numerous fine chemicals and specialized polymers, which triggered their broad expansion and fomented a vibrant global market, pointing to annual sales of over USD 2,000 million in the United States alone (Cunningham et al., 2020). PFOA and PFOS have been considered key representatives of this chemical class (Han et al., 2021), though they are being gradually phased out globally due to their persistence and confirmed human carcinogenicity and teratogenicity, and replaced by shorter-chain perfluorinated derivatives (Wallington et al., 2021). These smaller perfluorinated derivatives (e.g., perfluorobutanoic acid, perfluorobutanesulfonic acid or perfluorohexanoic acid) have acquired significant notability, more recently, due to their presumably safer toxic profile (Anderson et al., 2019; Luz et al., 2019) though their environmental persistence remains a worrying issue (Wallington et al., 2021).

The broadened spectrum pictured by the OECD revised definition of PFAS expands this chemical category by incorporating an array of different polyfluorinated compounds, most noticeably many pharmaceuticals and pesticides, which exhibit an incredible chemodiversity but also vastly different biological activities. Under this new rule, many of these newly defined PFAS have been on the market already for several decades. For instance, at least 14 of the most popular pesticides worldwide are classified as PFAS (Table 1) (McDougall, 2017; Maienfisch and Hall, 2004). While polyfluorination is a common feature in flagship pharmaceuticals, some trifluoromethylated high-grossing pharmaceuticals, such as fluoxetine or celecoxib, can also be encountered (Table 1) (Mykhailiuk, 2021; Ismail, 2002).

Moreover, the refurbished interest in polyfluorinated compounds has also led to the introduction of many new bioactive molecules containing perfluorinated elements, exponentially increasing the selection of PFAS currently approved for use. This trend is quite noticeable in the agrochemical industry, where a surge of heavily fluorinated agrochemical ingredients has recently been observed. Between 2015 and 2020, when over 70% of the newly approved pesticides containing at least one fluorine heteroatom, a gamut of pesticides now classified as PFAS were proposed to be introduced in the market and are awaiting approval in the EU (Alexandrino et al., 2022). Noticeably, many of these new agrochemical formulae detached from common fluorination strategies

Table 1
Examples of top-selling pesticides and pharmaceuticals included in the new PFAS definition.

Name	Molecular structure	Class	Launch year	Approval status in the EU
		Pesticides		
Haloxyfop		Herbicide	1980	Not approved
Pyroxsulam		Herbicide	2007	Approved
Picoxystrobin		Fungicide	2001	Not approved
Trifloxystrobin		Fungicide	1999	Approved
Fipronil		Insecticide	1992	Not approved*
Flubendiamide		Insecticide	1979	Approved
		Pharmaceuticals		
Fluoxetine		Antidepressant	1986	Approved
Mefloquine		Antimalarial	1980s	Approved
Halofantrine		Antimalarial	1980s	Approved
Celecoxib		Anti-inflammatory	1999	Approved
Sitagliptin		Antidiabetic	2006	Approved

* Approved only in Belgium and the Netherlands

(e.g., incorporation of a single CF_3 moiety), opting instead for approaches involving the incorporation of multiple perfluorinated moieties in their chemical design. For instance, the pesticides pyrifluquinazon and broflanilide (Fig. 1) contain a heptafluoroisopropyl moiety, with the latter chemotype harboring an additional CF_3 group and an arylic fluorine, totalizing 11 fluorine heteroatoms (El Qacemi et al., 2019). Another good example is the acaricide flupentiofenox (Fig. 1), introduced in 2020, which contains two perfluorinated groups bound to sulfur heteroatoms, namely a trifluoromethyl-sulfinyl moiety and another trifluoromethanethiolate functional group (Umetsu and Shirai, 2020). Likewise, during the second half of the last decade, fluorinated pharmaceuticals also exhibited a significant representation (up to 50%) in the gross volume of pharmaceuticals approved for use (Inoue et al., 2020). Of these, about 20% corresponded to pharmaceuticals holding perfluorinated motifs (Inoue et al., 2020), now considered PFAS in light of the revised definition. Yet, in clear contrast with the fluorination dynamics of agrochemicals, all these pharmaceuticals PFAS correspond to CF_3-bearing compounds, differing only in the site where fluorination occurs. For instance, berotralstat (approved in 2021) or apalutamide (approved in 2018) exhibit CF_3-substitution in heterocyclic moieties (Fig. 1), while esaxerenone (approved in 2019) and nirmatrelvir (approved in 2021) reveal arylic and aliphatic CF_3-fuctionalizations, respectively (Fig. 1) (Inoue et al., 2020; Yu et al., 2020).

3. The ubiquity of PFAS in the marine environment: sources and consequences

Awareness and knowledge of PFAS sources, accumulation and impact are key procedures to ensure environmental protection in terrestrial and sea/ocean ecosystems. Understanding these dynamics is particularly relevant for the marine environment as it constitutes to be the major environmental sink of legacy and newly defined PFAS (Zhang et al., 2019a). The various input sources of PFAS in the environment are mostly related to their production (e.g., fine chemistry industries), wide-scale utilization, and incorrect disposal (e.g., incomplete removal in wastewaters treatment plants, runoff of PFAS-containing leachates in landfills) (Brase et al., 2021). Since most of these PFAS sources are confined to inland activities, the introduction of these substances into the marine environment primarily happens through riverine discharges, while it is also known that volatile PFAS can be transported by the atmosphere (Zhang et al., 2019a). Despite the increasing reports on their harmful effects, there is still no adequate data on long-term monitoring or distribution of PFAS in marine ecosystems.

Another problem is that wastewater treatment may generate even more PFAS in effluents than in influents due to the generation of polyfluorinated metabolites (Houtz et al., 2018; Eriksson et al., 2017). The issues of sustainable waste treatment are particularly complex in

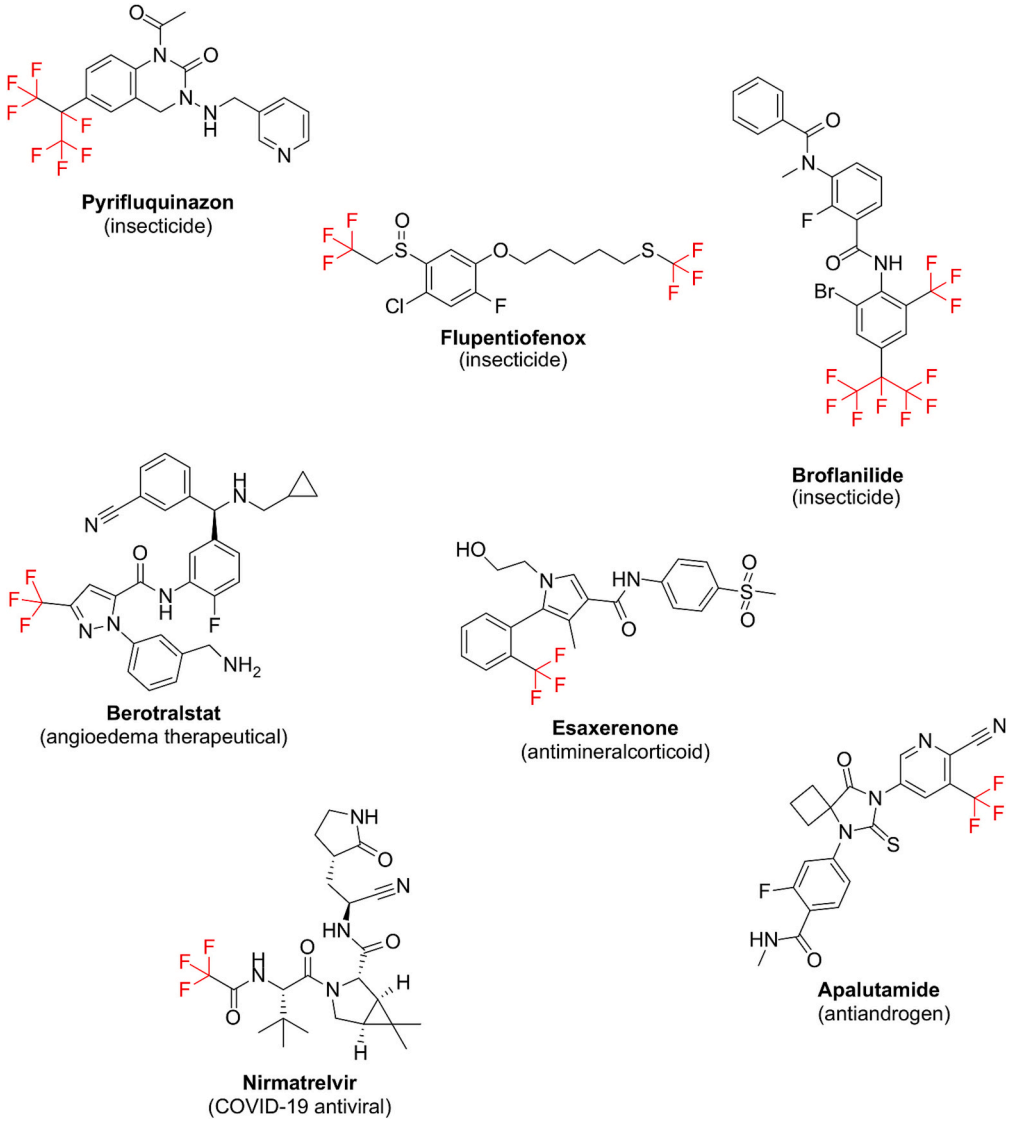


Fig. 1. Examples of pesticides and pharmaceuticals PFAS introduced in the market between 2015 and 2021.

emerging and developing countries, owing to poor or non-existing local management systems, widespread corruption, and a lack of interest in this subject by the governmental institutions. In addition, new compounds used as an alternative to the ones proven hazardous, whose effects are insufficiently known and may arise in future generations, are inevitable concerns related to the PFAS topic.

3.1. Climate change contributes to the marine distribution of PFAS

The global climate change affects modifications in the spatial distribution of chemical pollutants in the oceans, endangering previously unaffected species and their populations. Consequently, given that permafrost tends to significantly deteriorate and thaw in response to global warming, the glaciers and permafrost in the polar areas may become crucial pools of airborne PFAS (Mahmoudnia et al., 2022). It has been reported that short-chain PFAS are more mobile and water-soluble (Li et al., 2020a), and even more persistent (Brendel et al., 2018) than long-chain ones, so the freeze-thaw process of the active layer leads to higher concentrations of short-chain PFAS in deeper parts of permafrost and closer to groundwater than long-chain PFAS. This will undoubtedly pose a long-term threat to environmental health, especially for northern marine ecosystems, as well as to public health in northern countries due to the predominant reliance on these water bodies for producing drinking water (Lohmann et al., 2020).

PFAS compounds have been detected in numerous ecosystems and environmental compartments (Kurwadkar et al., 2022), including at high altitudes, in the snow and meltwater from Mt. Everest (Miner et al., 2021), as well as from Arctic ice and soil (Mahmoudnia et al., 2022). In detail, from the 14 PFAS compounds analyzed from the Khumbu Glacier (Mt. Everest), PFOS, PFOA and perfluorohexanoic acid (PFHxA) were detected in samples from Everest Base Camp, Camp 1, Camp 2, and Everest Balcony, using solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Miner et al., 2021). In addition to these atmospheric depositions, rivers are considered to be the primary inputs of PFAS into marine ecosystems, as recently confirmed by inverse solid correlations between salinity and concentrations of the majority of PFAS compounds in the coastal part of the Northwestern Atlantic, indicating that continental discharges constitutes a significant source of marine pollution (Zhang et al., 2019a). Thus, PFAS compounds enter the oceans in significant quantities and, like other persistent chemicals, they are introduced in the marine food chain, ultimately reaching humans (Miranda et al., 2021a).

The Atlantic Ocean is an example of a marine ecosystem highly impacted by PFAS, with these compounds showing a multilayered distribution stretching from the Atlantic atmosphere (Wang et al., 2015a) to the deeper layers of its water column (Miranda et al., 2021b). PFAS predominant occurrence and distribution has significant repercussions on its wildlife, from primary producers and consumers (i.e., plankton) (Zhang et al., 2019a) to apex predators (e.g., shearwaters or dolphins) (Escoruela et al., 2018; Houde et al., 2006). Perfluoroalkyl carboxylic acids (e.g., PFOA, PFOS) have been the principal target of the screening programs, with these compounds usually displaying background concentrations at the pg/L level (Miranda et al., 2021b), although a reported study focused on the northern sector of the Atlantic Ocean reported concentrations of this subtype of PFAS reaching ng/L concentrations (Zhao et al., 2012). Likewise, several pesticide PFAS, such as trifluralin and λ-cyhalothrin, have also been detected in estuaries connecting to the Atlantic Ocean at ng/L concentrations (Cruzeiro et al., 2017; Cruzeiro et al., 2016).

3.2. PFAS bioaccumulation and biomagnification on marine ecosystems

Upon arrival in the ocean, legacy and newly defined PFAS have a lasting effect on marine organisms across all trophic levels through bioaccumulation and biomagnification. These two processes are part of the same scenario of pollutant accumulation in marine biota, where bioaccumulation occurs at lower trophic levels due to direct uptake of PFAS from contaminated seawater and sediments (PFAS are mostly present in water or contaminated sediments), followed by biomagnifications that refers to an increase in the concentration of pollutants in the tissues of organisms at successively higher trophic levels, primarily threatening apex predators. Many PFAS tend to be bioaccumulative (Jahnke and Berger, 2009), with this bioaccumulation potential increasing with carbon chain length (Spaan et al., 2020). To illustrate this, PubMed search on key words "PFAS, bioaccumulation, marine" for the last five years resulted with 40 publications related to bioaccumulation in marine organisms, out of a total of 343 publications including different aquatic ecosystems (Suppl. Fig S1A). In addition to the legacy PFAS, whose ability to bioaccumulate has been well documented in numerous studies (Cara et al., 2022; Langberg et al., 2019; Martín et al., 2019), data for newly defined PFAS have been increasing in recent years (Munoz et al., 2022); e.g for GenX (HFPO-DA), the current-use alternative to PFOS and PFOA, it was recently reported to have equal or even greater toxicity and bioaccumulation capacity than PFOA (Yang et al., 2022).

It has been reported that sediments, not water, are the primary source of PFAS in the benthic food webs in the lakes of the Canadian High Arctic (Lescord et al., 2015). Likewise, sediments are crucial for understanding the fate of PFAS in the Arctic, as they represent a key reservoir and, thus, the major input of PFAS for the benthic food web (Lin et al., 2020). The recently studied bioaccumulation dynamics of six selected PFASs in the sea urchin *Holothuria tubulosa* showed a higher concentration in its tissues (primarily in the intestines, then in the gonads) than in the surrounding sediment and seawater. Furthermore, due to the strong adsorption of these compounds on sediments, mean concentrations of selected PFAS were higher in sediments than in water. These studies provided a clear view of the fate and distribution of PFAS in the marine environment, emphasizing that the bioaccumulation at lower trophic levels is a potential source of contamination of organisms at higher trophic levels (Martín et al., 2019).

One of the first studies about PFAS bioaccumulation in plankton samples from the oligotrophic global ocean, including tropical and subtropical Pacific, Atlantic and Indian Ocean, showed a selective fractionation of branched PFAS in the ocean surface, mediated by plankton (Casal et al., 2017). Namely, branched isomers of PFAS, formed as by-products of PFAS production by electrochemical fluorination, in addition to the intended linear isomers, represent a large amount of PFAS load in the environment. Due to structural higher polarity, branched isomers remain in seawater, while linear PFAS bind to sediments. This fact may explain why most marine animals preferentially accumulate the linear PFAS isomers (through the food web). At the same time, humans appear to preferentially accumulate the branched isomers (by consumption of the fish) as these are often found in human serum (Kurwadkar et al., 2022; Schulz et al., 2020). Also, the vertical transport of PFAS, due to their sorption to organic matter, results in a wide variability of PFAS incidence time on the ocean surface (Casal et al., 2017). These findings suggested potentially different toxic effects on pelagic, compared to bentopelagic and benthic fish populations, which may harm their consumers, including humans. Indeed, small pelagic fish are considered key components of marine ecosystems, affecting population dynamics, particularly in highly productive marine upwelling systems, but also making up to 25% of fish landings intended for human consumption (Queiros et al., 2019).

In addition to bioaccumulation, predation is another mechanism that magnifies PFAS through the marine food web (Zhang et al., 2019a). Marine apex predators, such as sharks, tunas and marine mammals are particularly susceptible to bioaccumulation and biomagnification phenomena (Houde et al., 2006), owing to their top position in the marine food chain and long lifespan (Sciancalepore et al., 2021). Due to their long-term incidence in some localities (high level of site fidelity) (Díaz López, 2019), dolphins, in particular, can serve as important sentinels of the health of coastal marine ecosystems (Wells et al., 2004). Twenty

years ago, it was proven that marine organisms, particularly seabirds and marine mammals, accumulate harmful concentrations of "forever chemicals" (Kannan et al., 2002). Over the last decade, special attention has been paid to the top of the food chain by studying the hazards posed by PFAS to sharks (Chynel et al., 2021; Zafeiraki et al., 2019) and marine mammals, as shown by an increasing number of recent ocean studies around the world (Barrett et al., 2021; Sciancalepore et al., 2021; Stockin et al., 2021; Ning et al., 2020; Lynch et al., 2019; Fujii et al., 2018; Dassuncao et al., 2017; Galatius et al., 2013). Naturally, this trend has also extended to numerous fluorinated pollutants, now classified as PFAS, many of which are ubiquitous marine pollutants and highly involved in bioaccumulation/biomagnification occurrences (Alexandrino et al., 2022). For instance, several pyrethroids harboring perfluorinated elements (e.g., λ-cyhalothrin, bifenthrin) were found to accumulate in fish and dolphins, often corresponding to the most frequently detected pesticides in their corresponding screening panels (Clasen et al., 2018; Aznar-Alemany et al., 2017; Alonso et al., 2012).

3.2.1. Health of marine wildlife associated with PFAS exposure

Immune dysfunction was one of the first well-studied harmful effects of PFAS, mainly due to bioaccumulation and exposure to multiple persistent chemicals, such as PFOA and PFOS, reviewed by DeWitt et al. (DeWitt et al., 2011). This reported study, which included laboratory rodent models, wildlife (fish, reptiles, seabirds, marine mammals) and human samples, revealed the impact of PFOA and PFOS on the adaptive and innate immune response as on the inflammatory response through cytokines expression. Subsequent findings implied the PFOS capability for chronic immune activation in the bottlenose dolphin (*Tursiops truncatus*) due to increasing CD4+ and CD8+ T cell proliferation and promotion of proinflammatory cytokine production such as interferon-gamma (IFNγ), but not immunoregulatory interleukin-4 in T-cells (Soloff et al., 2017). This adverse impact on dysregulation of the cellular immune system due to PFOS exposition has numerous consequences, such as hepatotoxicity, neurotoxicity and developmental disorders (Fair et al., 2013; Lau et al., 2007; Lau et al., 2004). Moreover, it has already been reported that the immune system appears particularly vulnerable to PFOS and other perfluoroalkyl acids (PFAA) exposure compared to other toxicological endpoints (Corsini et al., 2014; Dewitt et al., 2012). A recent evaluation of liver bioaccumulation of 17 targeted PFAS in bottlenose dolphins from the northern Adriatic sea showed PFAS profiles composed mainly of the same five dominant compounds, with PFOS prevalence, followed by perfluoroundecanoic acid (PFUnA), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA) and perfluorotridecanoic acid (PFTrDA). Notably, the authors of this study emphasize the relevant role of Environmental and Tissue Banks for retrospective analyses of emergent pollutants (Sciancalepore et al., 2021), which could excellently support the monitoring of PFAS bioaccumulation in marine mammals. Concerning immunotoxicity, it has been reported that high concentrations of PFOA in the liver of the southern sea otter (*Enhydra lutris*) from the California coast are associated with increased mortality from infectious diseases (Kannan et al., 2006), but also gastrointestinal parasite infestation of different animal taxa, especially apex predators of the marine food chain (Carravieri et al., 2020). Surprisingly, immunotoxicity has also been highlighted as a common ecotoxicological endpoint of newly defined PFAS, particularly PFAS pesticides and pharmaceuticals (Alexandrino et al., 2022). For instance, Mirghaed et al. (Taheri Mirghaed et al., 2020) have shown that the PFAS pesticides flonicamide and lunefuron trigger immunosuppression phenotypes in the common carp (*Cyprinus carpio*), primarily by causing oxidative stress via the impairment of antioxidant-related genes. As for pharmaceutical compounds harboring perfluorinated elements, fluoxetine has proven to be a representative example of these impacts, given the substantial reports of its immunotoxic potential towards various marine organisms, particularly in the bivalves *Mytilus edulis* (Lacaze et al., 2015), *Tegillarca granosa* (Shi et al., 2019), or *Venerupis philippinarum* (Munari et al., 2014).

The factors controlling the bioaccumulation and tissue distribution of different PFAS are not fully elucidated. However, many PFAS are ionic and, unlike neutral hydrophobic organic contaminants, they are thought to accumulate in phospholipids and protein-rich tissues, as shown in a study with the long-finned pilot whale (*Globicephala melas*) from North Atlantic (Dassuncao et al., 2019). For example, PFAA is anionic under certain environmental conditions and predominantly accumulate in blood and liver, as shown in wild animals and humans (Domingo et al., 2012; Lau et al., 2007). In the past decade, it was reported the strong interaction of short-chain PFAA with human serum albumin (HSA). This major serum protein likely contributes to its tissue distribution and bioaccumulation patterns (Bischel et al., 2011). Forsthuber et al. (Forsthuber et al., 2020) recently reported that HSA is the primary transport protein for PFOS, PFOA, perfluorononan-1-oic acid (PFNA), perfluorohexanesulfonic acid (PFHxS) and PFDA, as well as PFAA. The accumulation of these compounds in the albumin fraction of plasma implies that they have similar binding mechanisms and can be transported throughout the body in the same manner as fatty acids (van der Vusse, 2009).

Furthermore, the liver fatty acid binding protein (L-FABP) efficiently binds PFAS. It shows a high affinity for binding long-chain fatty acids and their oxidation products (Zhang et al., 2013). Still, unlike other fatty acid-binding proteins, it can bind more than one molecule of long-chain fatty acids and a range of ligands (De Gerónimo et al., 2010). Due to its fatty acid-like structure, PFAA may efficiently compete with fatty acids and bind with these natural ligands for L-FABP. PFAA also interferes with the binding of fatty acids to their transporters, which can disrupt the regulation of lipid metabolism (Sheng et al., 2016). Therefore structural similarities between PFAS (particularly PFOS, PFOA, PFHxS, PFNA and PFDA) and fatty acids have become crucial in understanding their distribution and bioaccumulative potential (Forsthuber et al., 2020).

Although the brain is not a dominant tissue for PFAS accumulation compared to blood and liver, adverse effects on brain functions have been reported, including those associated with alternation in brain steroid hormones concentrations in East Greenland polar bear (*Ursus maritimus*) (Pedersen et al., 2016). As steroid hormones play an essential role in brain plasticity and sex-specific behavior based on sexually dimorph brain function (Remage-Healey and Bass, 2007), such negative effects may soon be expected in other marine mammals. Importantly, *U. maritimus* is considered as one of the leading sentinel species, given its similar physiology to humans, as well as its long lifespan and thus long exposure to pollutants, like humans (Bossart, 2011).

In a recent review, Cao and Ng (Cao and Ng, 2021) summarized their findings on two potential mechanisms of PFAS entering the brain: initiating blood–brain barrier (BBB) disassembly through disrupting tight junctions and relying on transporters located at the BBB. The BBB is a crucial immunological feature of the central nervous system (CNS), composed of brain endothelial cells connected by tight junctions, which protects neurons by maintaining brain homeostasis. Its dysfunction amplifies a neuroinflammation (Takata et al., 2021), which plays a significant role in the onset and progression of a wide range of human neurodegenerative disorders (Guzman-Martinez et al., 2019). Marine mammals, and even birds, could be exposed to such a scenario, where the relative abundance of PFAS in the brain significantly increases according to carbon chain lengths, implying that long-chained PFAS, compared to the short-chained, may cross the BBB much more efficiently (Dassuncao et al., 2019). Additionally, several recent studies have shown that elevated PFAS levels in different parts of the *U. maritimus* brain affect the upregulation of oxidative stress mechanisms (Eggers Pedersen et al., 2015) and the rising serum C-reactive protein, implying that inflammation facilitates PFAS penetration across the BBB (Wang et al., 2018).

A recent comprehensive review by Starnes et al. (Starnes et al., 2022) documents that exposure to long-chain PFAS in adult animal models may affect neurobehavioral functions, such as spatial learning and

memory, as well as motor activity and coordination. These neurobehavioral and cognitive effects, probably caused by changes in sex hormone levels, are more pronounced in adolescents than in the elderly (van Larebeke et al., 2022), opening numerous new questions and concerns in the behavioral biology of various marine animals. Kim et al. (Kim et al., 2020) showed that exposure to 8:8 perfluoroalkyl phosphinic acid (8:8 PFPiA) induces developmental neurotoxicity and alters epigenetic mechanisms in early life stage zebrafish *Danio rerio*, possibly due to disruption of thyroid hormones and inhibition of neuronal development.

The transferring of organic contaminants into offspring during reproduction or gestation referred to as maternal offloading (Wood et al., 2021), has also been observed for PFAS. In endangered species of sea turtles on remote Pacific islands, which contained high levels of PFAS in their blood, the concentration of this pollutant in their eggs was recorded at levels harmful to birds (Wood et al., 2021). Additionally, a recent study provided valuable biochemical insight into the impacts that maternal offloading and bioaccumulated PFAS transference have on oviducal eggs of freshwater turtle (*Emydura macquarii macquarii*), where yolk comprised >90% of the bioaccumulated PFAS load (Beale et al., 2022c). Here, multi-omics analysis of the dissected egg fractions showed elevated histidine metabolism, potentially associated with metabolic dysfunction and growth retardation (Moro et al., 2020), as well as significantly elevated purine metabolism, which has been observed in fertile chicken eggs infected with *Salmonella* Hessarek, probably linked to stress response during infection (Lin et al., 2021). Besides purine nucleotides are building blocks of DNA and RNA, energy sources and enzyme cofactors in metabolic pathways, they also play an important role in the cell survival and proliferation (Yin et al., 2018), in addition to the immune response and host-tumor interactions (Di Virgilio and Adinolfi, 2017).

Furthermore, microbial community profiling of the *E. macquarii macquarii* feces, indicated a shift in the ratio of Bacillota (= Firmicutes *corrig. Gibbons and Murray, 1978*) to Bacteroidetes (B:B), which is known to be a significant biomarker of health in animal models and clinical studies (Beale et al., 2022a; Beale et al., 2022b). In contrast, the potential correlation between PFAS-induced hepatotoxicity and gut bacterial community was recently reported for the black-spotted frog (*Rana nigromaculata*) (Lin et al., 2022). These health-related consequences of gut microbiota imbalance, or gut dysbiosis, which mainly affect immunity, metabolic and neurological functions (Balaguer-Trias et al., 2022; Chiu et al., 2020) raise new challenges in ecotoxicology and the wildlife welfare and conservation. In addition to the adverse impact on microbial biodiversity, especially in contaminated sites (Senevirathna et al., 2022), the long-term effects of PFAS on (genetic, species, ecosystem and functional) biodiversity will be even more pronounced, with epigenetic modifications which lead to alteration in gene expression, reviewed by Kim et al., including mammalian and aquatic model organisms (Kim et al., 2021).

3.2.2. Insights of PFAS effects on human health

The consumption of seafood (Sunderland et al., 2019; Wang et al., 2015b), marine mammals in northern communities (Tomy et al., 2004) and, in some cases, of drinking water (Li et al., 2022a; Hammarstrand et al., 2021; Domingo and Nadal, 2019) has been considered a major source of legacy PFAS exposure in humans. These compounds can enter the food chain directly by ingesting contaminated food or indirectly through food contact materials (FCM) (Ramírez Carnero et al., 2021). FCM, defined as *materials intended to come in contact with food during its transport, storage, conservation, handling, or manufacture* (EC Regulation, 2011), are widely used in the food industry and household food storage, and include cutlery, crockery, dishes, containers, processing machines, cutting boards, etc. (Karamfilova, 2016). In terms of newly defined PFAS, human exposure to these compounds is most associated to the ingestion of crops treated with PFAS pesticides and to occupational exposure to these molecules (Alexandrino et al., 2022), as well as through the ingestion of seafood. For instance, pyrethroid pesticides harboring perfluorinated elements, such as λ-cyhalothrin, have been detected in human breast milk (Feo et al., 2012; Sereda et al., 2009), which has shown to manifest into a gamut of deleterious effects, particularly endocrine disruption and reproductive toxicity in children and adults (Orton et al., 2011; Oh et al., 2007).

Due to the ability of PFAS to disrupt hormone metabolism by mimicking fatty acids, they are considered endocrine-disrupting chemicals (EDCs) (Gore et al., 2015). Their negative impact is primarily manifested in thyroid function (De Toni et al., 2022; Coperchini et al., 2021), especially during pregnancy (Derakhshan et al., 2022; Jensen et al., 2022), whose hormones are crucial for numerous metabolic processes but also normal brain development, including neurogenesis, synaptogenesis and myelination. PFAS exposure has harmful effects on reproductive health (Hærvig et al., 2022; Petersen et al., 2022; Rickard et al., 2022a; Hammarstrand et al., 2021) breastfeeding reducing (Criswell et al., 2020), decreasing infant birth weights (Shoaff et al., 2018) with significant genotoxic potential that may result in congenital disabilities (e.g. anogenital distance in male newborns (Tian et al., 2019)), as well as delayed development and developmental neurotoxicity (Gaballah et al., 2020) with increased risk of autism spectrum disorder (ASD) (Oh et al., 2021). PFAS impact on alterations in the early-life gut microbiome development was recently reported for newborns (Naspolini et al., 2022) followed by decreases in gut microbiota richness in young children (Gardner et al., 2021).

Numerous experimental studies highlighted the harmful impact of elevated PFAS levels, particularly PFAAs, on immune system suppression (von Holst et al., 2021; DeWitt et al., 2019), resulting in a decreased antibody response to vaccines (Grandjean et al., 2017) and infectious disease resistance (NTP, 2016). In addition to legacy PFAS, such as PFOS and PFOA, with proved immunotoxicity, a recent study showed that exposure to PFAS alternatives, such as chlorinated polyfluorinated ether sulfonic acids (Cl-PFESAs) and perfluorobutanoic acid (PFBA) was associated with lower hepatitis B antibodies (HBsAb) in adults (Zeng et al., 2020). It is also warned that PFAS could reduce the effectiveness of the new coronavirus vaccines, according to toxicological studies proving the connection between the exposure to PFAS and the development of severe Covid-19 infections (Grandjean et al., 2021). In this case, the deterioration of clinical features could be explained by the fact that exposure to PFAS may lead to acute pulmonary toxicity due to inhibition of pulmonary surfactant function, followed by modulation of the pro-inflammatory response in bronchial epithelial cells (Sørli et al., 2020). The number of studies on the impact of PFAS on the development of malignant neoplasms (Boyd et al., 2022; Li et al., 2022a; Messmer et al., 2022; Vieira et al., 2013), mainly reproductive organs (Hu et al., 2022; Imir et al., 2021) and breast cancer (Wan et al., 2022; Itoh et al., 2021), as well as resistance to platinum-based chemotherapy (Rickard et al., 2022b) has significantly increased over the past five years. Thus, PubMed search on key words “PFAS, cancer” resulted with 123 publications, of which 58 were listed in the past 12 months (Suppl. Fig S1B).

Nevertheless, the most worrying issues are the possible interactions between environmental contaminants and/or pollutants, which can act simultaneously, even synergistically, in terms of their harmful effects on living organisms. In a comprehensive review, Chiu et al. (Chiu et al., 2020) summarized current knowledge on major classes of environmental chemicals, such as persistent organic pollutants, including PFAS, heavy metals, bisphenols, phthalates and pesticides and their effects on the human gut microbiome, including changes in microbial composition, gene expression, and impact on the host homeostasis.

4. PFAS associated with microplastics: A new concern of a forever alliance

Pollution of marine ecosystems by PFAS is of particular concern because these compounds survive for several decades in the ocean, can be transported over long distances (Garcia-Barrios et al., 2021), and act

in interactions with other pollutants, like MPs which are also a class of highly stable anthropogenic environmental pollutants commonly found in aquatic environments, wildlife, and humans. They also represent a diverse group of contaminants, and the synergistic effect of these pollutants with PFAS is particularly concerning. Bakhshoodeh and Santos (Bakhshoodeh and Santos, 2022) analyzed the published scientific records from 1990 to 2020 intending to visualize changes in bibliometric and scientometric trends in these two hot topics. They noticed that, in recent years (starting in 2018), studied topics linked to MPs had surpassed those related to PFAS, resulting in a significant increase in the MPs/PFAS ratio from 0.2 in 2011 to more than 2.5 in 2020, as well as that PFAS research topics were more prevalent in countries with larger areas of water than land (Bakhshoodeh and Santos, 2022).

4.1. Microplastics in marine environment: Deliberations and standing facts

The problem of the profound negative impact of MPs on marine ecosystems is growing with macroplastics deposits in the sea, which will remain there for centuries (GESAMP, 2015) due to their meager biodegradability rate (Ügdüler et al., 2020; Raddadi and Fava, 2019) and accessible entrance into the food chain. The lack of proper waste management leads to the accumulation of up to 80% of plastic waste each year in the ocean, which, scattered by waves, currents, winds and UV light, transforms it into microplastics and nanoplastics (NPs), spreading across the ocean and coastal areas, resulting in serious social, health, economic and environmental consequences (Oliveira et al., 2020, 2022; van Sebille et al., 2020).

In 2019, 6.1 Mt of plastic waste leaked into the aquatic environments and 1.7 Mt flowed into the oceans[2]. Besides macroplastics (> 5 mm), marine litter also includes MPs (5 mm - 1 μm), and NPs (< 1 μm) (Hidalgo-Ruz et al., 2012; da Silva et al., 2020) as an emerging pollutant (Park and Kim, 2019; Zhang et al., 2019b). For more information, see section SI1: Supplementary Information on Microplastics.

4.2. The interaction of MPs and PFAS

Different PFAS were detected and assessed, for the first time, from beached plastic debris and sediments collected in Greek coastal areas, with plastic pellets generally presenting a higher concentration of PFAS (in the range of 10 to 180 ng/kg) than sediments from the same sampling sites (Llorca et al., 2014). Additionally, MPs with adsorbed PFAS have been detected in other aquatic environments, such as river estuaries (Cheng et al., 2021) and lakes, where adsorption of PFAS by MPs is greatly enhanced by the presence of inorganic and/or organic matter and may present an environmental hazard for aquatic biota (Scott et al., 2021).

MPs' ability to adsorb PFAS depends on many factors such as type of plastic, type of PFAS and its molecular structure, exposure duration and conditions (i.e., aqueous salinity, temperature, and pH). The main forces responsible for the interactions of MPs and PFAS are electrostatic and hydrophobic, with hydrogen and covalent bonds being less important (Joo et al., 2021; Gagliano et al., 2020; Du et al., 2014). PFAS adsorption to MPs has been experimentally tested by Llorca et al. (Llorca et al., 2018) using a set of three types of common MPs, comprising of high-density polyethylene (HDPE), polystyrene (PS), polystyrene carboxylate (PS-COOH), and 18 PFAS (including carboxylic acids, sulphonates and one sulphonamide) from the surrounding waters (freshwater and seawater). PFAS adsorption was greater to PS and PS-COOH than to HDPE. Furthermore, adsorption kinetics was higher for MPs of smaller diameters. In the aquatic environment, minor MPs can be more readily ingested by various organisms; hence MPs with smaller size can promote the transfer of MPs and PFASs to the marine food chain and eventually reaching humans (Llorca et al., 2018).

Although PFAS adsorption to MPs has been detected in the aquatic environment and recreated in laboratory conditions (Hartmann et al., 2019), the obtained results may be significantly underestimated since most of these studies were based on using intact/pure polymers from commercial suppliers. In this regard, Ateia et al. (Ateia et al., 2020) questioned whether pure polymers should be used as surrogates for commercial MPs in pollutant adsorption models and then experimentally demonstrated that pure polymers (pure MPs) had lower uptake values than MPs with plasticizing agents, such as phthalates, in general. In contrast, the difference in commercial MPs samples was explained by surface roughness and/or the presence of fillers (e.g. talc and glass fiber) (Hartmann et al., 2019; Hartmann et al., 2017). Therefore, since MPs can form complexes or co-adsorb with natural organic matter and harboring microbial biofilms, further increasing the intake of micropollutants (Scott et al., 2021; Amaral-Zettler et al., 2020), it has become apparent that the use of real MPs in research is crucial to obtain ecologically relevant results, and that the assessment of MPs sorption without natural organic matter may result in the underestimation of their actual values (Ateia et al., 2020).

4.2.1. Harmful impact of synergy between MPs and PFAS on living organisms: Growing concern for aquatic biota

Even though MPs and PFAS have each had their occurrence and fate extensively studied, little is known about how the two are connected and how they overlap. In 2023, Kang et al. (Kang et al., 2023) provided a thorough understanding of how MPs and PFAS interact, overlap, and have toxic effects when present in the environment. MPs and PFAS have co-sources like materials that come into contact with food and textiles with practical uses in daily life. Thus, it is crucial to control PFAS and MPs at the source because these are frequently found together in soil, air, and water bodies and are highly correlated with human activity and population density. PFAS can bind to MPs, through two main sorption mechanisms, electrostatic and hydrophobic interactions, which may be affected by the environmental conditions, and MP and PFAS properties. The presence of MPs may affect how PFAS are transported and degraded in the environment. Their global distribution may also be impacted by ecological factors like atmospheric deposition, rainwater drenching, runoff, wave currents, and tides, among others (Scott et al., 2021).

Although data and estimates on the actual impact of MPs on the transfer of PFAS to the aquatic food chain are limited, it is known that MPs can serve as a vector for PFAS transfer to soil, boosting PFAS uptake by earthworms (Lumbricina) which results in increased toxicity (Sobhani et al., 2021). The same effect may be observed in marine organisms. In recent study, Islam et al. (Islam et al., 2021) showed that MPs act as a vector of PFOS in the clam *Scrobicularia plana* tissues, although PFOS accumulation is independent of MP size. Synergy between MPs and PFAS particularly affects fish (Savoca et al., 2021), and thus potentially human and wildlife health, as recently reviewed by several authors (Rodríguez et al., 2022; Aryal et al., 2020; Campanale et al., 2020; Sharma and Chatterjee, 2017). It is known that ingestion of (micro) plastics can be life-threatening due to intestinal obstruction, gastrointestinal inflammatory response and consequent reduction in nutrient absorption and this especially endangers sea turtles (Camedda et al., 2022; Rodríguez et al., 2022; Choi et al., 2021), sea birds and mammals (Robuck et al., 2022; Senko et al., 2020). Moreover, a study by Tamargo et al. (Tamargo et al., 2022) on the alteration of human microbial colonic communities caused by ingestion of MPs, suggests that some members of the colonic microbiota could adhere to MPs surface, promoting the formation of biofilms, which implies severe consequences for gastrointestinal health, increasing the risk of allergic reactions, type 2 diabetes, (Patil et al., 2022), obesity (Zhao et al., 2022; Kannan and Vimalkumar, 2021) cancer (Kumar et al., 2022; Sharma et al., 2020; Yan et al., 2020), and even resistance to chemotherapy in gastric cancer (Kim et al., 2022). In addition to the detrimental effects on gut homeostasis,

[2] https://www.oecd.org/environment/plastic-pollution-is-growing-relentlessly-as-waste-management-and-recycling-fall-short.htm

resulting in upregulated oxidative stress and increased inflammatory processes, the impact of PFAS on intestinal microbiota can be durable and carried over to the next generation, as shown on the fish marine medaka *(Oryzias melastigma)*, which further increases concerns about the synergistic action of these two pollutants in the digestive tract (Chen et al., 2018).

To date, approximately 4,028 species (1,924 publications) have been reported to be affected by marine debris, of which as much as 68% is littered with various plastic materials (https://litterbase.awi.de/interaction_detail; date of access, 28 October 2022). The harmful effects of MPs in the marine environment are numerous and well documented (Nabi et al., 2022), while their hazardous impact on wildlife in association with PFAS remain to be adequately elucidated. Sediments mixed with plastics show changes in heat transfer, resulting in lower residues, maximum temperatures and slower heating. These alterations could have many consequences for beach organisms, including those whose sex is determined by temperature, such as sea turtles and other reptiles' eggs (Carson et al., 2011). In addition to recent studies on maternal offloading and bioaccumulated PFAS on oviducal eggs of marine and freshwater turtles (Beale et al., 2022c; Wood et al., 2021), these findings highlight the importance of adequate and effective management in marine protected areas (MPAs) to ensure the conservation of endangered marine reptiles.

In terms of the potential health impacts of MPs and unforeseen synergistic effects of these with PFAS, about which our knowledge is still scarce, a few recently published studies are particularly significant. Kwon et al. (Kwon et al., 2022) have shown that MPs, particularly those with a diameter of 2 μm or less, begin to accumulate in the brain within seven days of ingestion, causing apoptosis and changes in immunological and inflammatory responses. Thus, it was shown in a mice model for the first time that MPs infiltrate blood-brain barrier (BBB), which offered new insights into its possible role in neurotoxicity. In a pioneer study performed by Leslie et al. (Leslie et al., 2022), four high-production volume polymers used in plastic materials were identified and quantified in the human blood of healthy donors. Also, for the first time, MPs were detected by Raman microspectroscopy in all human placental portions (maternal, fetal and amniochorial membranes) (Ragusa et al., 2022; Ragusa et al., 2021), which may alter numerous molecular signaling pathways in the placenta and lead to adverse pregnancy outcomes, including preeclampsia and fetal growth restriction (Ilekis et al., 2016). The aforementioned studies were conducted on a mammalian model (mice) and humans, which directly indicates the possibility of a similar scenario in marine mammals and harmful effects due to the passage of MP through the BBB and the placental barrier. A new matter of concern is the possibility of MPs particles acting as vectors for PFAS transmission and faster expression of harmful effects on susceptible tissues, especially regarding the passage of BBB accompanied by increased neurotoxicity. We can assume that the numerous already known harmful effects of MPs and NPs will be even more expressed in synergy with PFAS and other persistent pollutants in the marine environment, whereby all ocean dwellers can be exposed to such a scenario.

5. (Bio)degradation of PFAS: recent discoveries, challenges of bioremediation and possible degradation pathways

5.1. Biodegradation of legacy and newly defined PFAS

Unlike many halogenated organic pollutants, the influence of environmental microorganisms in the fate and dynamics of PFAS in marine ecosystems is expected to be minimal, given their refractory nature. While for legacy PFAS, this has been long recognized, as shown by their colloquial connotation as "forever chemicals", newly defined PFAS are also not expected to be readily biodegradable. While the overall recalcitrance of PFAS is a direct consequence of their high fluorination degree, their resistance to biodegradation can also be attributed to the incredibly high fitness cost (i.e., the difficulty of microorganisms to draw energy from PFAS due to challenging thermodynamics) of their productive microbial catabolism (Wackett and McMahon, 2021).

5.1.1. High fitness cost of PFAS biodegradation

The microbial consumption and transformation of PFAS demands a significant investment of energy and resources, representing a considerable metabolic burden for potentially degrading microorganisms (Wackett and McMahon, 2021). Alongside their xenobiotic nature, environmental microorganisms cannot benefit from drawing energy from PFAS, as their typically low redox potential makes them not readily functionalized by microbial enzymes (Sun et al., 2021). As such, the emergence of suitable catabolic strategies based on the enrichment and selection of competent environmental microorganisms is expected to be a prolonged process, precluding the emergence of natural attenuation dynamics that could act as buffers to mitigate PFAS pollution in marine ecosystems. This has been clearly demonstrated by the many studies on the biodegradation of different PFAS, which almost consensually report inefficient catabolic processes, with many involving only a single degradation step (Zhang et al., 2022; Shahsavari et al., 2021).

Furthermore, the degradation of PFAS is also associated with their unusual chemical features, namely their hydrophobicity, chemical complexity and large molecular sizes, which muster various physiological challenges to microorganisms. On the one hand, the hydrophobicity and large molecular sizes of PFAS turn them into compounds with reduced bioavailability and whose cellular uptake demands the involvement of specialized active transport pathways (Wackett and McMahon, 2021). Not only these transmembrane apparatuses are narrowly distributed in most environmental microorganisms, but their recruitment also entails a significant investment of energy, further aggravating the energetic deficit associated with the microbial consumption of PFAS. For polyfluorinated pesticides, for example, the involvement of specialized membrane transporters, such as TonB-dependent or tripartite ATP-independent transporters, have been highlighted as critical components of the global catabolic strategy of degrading microorganisms (Alexandrino et al., 2021). Accounting for the reduced water solubility of PFAS, the intervention of surfactants has also shown to be an essential factor improving their bioavailability and, concomitantly, their biodegradation in the ecosystems (Bolan et al., 2021). On the other hand, the fluorination stoichiometry of PFAS suggests that their eventual mineralization would lead to a surge of fluoride ions swarming the cytosolic environment of degrading microorganisms. Given the cytotoxic nature of fluoride (Johnston et al., 2020; Ji et al., 2014) an increment of the cytosolic concentration of this anion would have to be matched by the expression of efficient efflux systems that could rapidly export fluoride to the extracellular environment. While several microbial fluoride export proteins have been characterized before (Berbasova et al., 2017; Ji et al., 2014; Li et al., 2013; Stockbridge et al., 2014), much is yet to know regarding their efficiency in assisting the microbial degradation of heavily fluorinated compounds.

5.2. PFAS defluorination is unlikely, but microbially feasible

The efficient cleavage of C-F bonds has long been recognized as a critical bottleneck in the biodegradation of fluoroorganic compounds, including PFAS (Zhang et al., 2022). Fluoride removal dismantles the prominent influence of this heteroatom on the chemical stability of an organic molecule, a direct result of its extreme electronegativity, while also improving the likelihood of the resulting metabolites being more readily degradable (Kiel and Engesser, 2015). For the case of bioactive PFAS, such as pharmaceuticals and pesticides, fluoride elimination has also been associated with the mitigation of their biological activities and, thus, a reduction of their potential ecotoxicity (Alexandrino et al., 2021; Carvalho et al., 2016). As such, C-F cleavage is a pivotal catabolic reaction for the effective biodegradation of PFAS. However, these reactions are rare catabolic events during the microbial degradation of both legacy and newly defined PFAS, with most studies revealing their

various shortcomings. For instance, the intervention of microorganisms on the environmental breakdown of 8:2 fluorotelomer alcohol often leads to the production of smaller perfluorinated carbon chains conserving all its fluorinated elements (e.g., perfluorohexanoic acid) (Wang et al., 2009). Similarly, biodegradation studies focused on perfluoroalkyl acids or aromatic PFAS often report substantial removal efficiencies without fluoride release, suggesting that preferably minor polyfluorinated compounds result from these biotransformations (Li et al., 2020b; Henning et al., 2019; Beškoski et al., 2018; Uniyal et al., 2016; Kwon et al., 2014). These typically inefficient biodegradation processes largely contribute to the release of fluorinated subproducts (Fig. 2), which may have significant environmental impacts. Howard and Muir (Howard and Muir, 2010) pioneered this chain of thought by highlighting hundreds of organic compounds and their metabolites, many qualifying as PFAS, that were not flagged as persistent in the appropriate regulatory frameworks, despite their prolonged environmental half-live values. More recently, Nascimento et al. (Nascimento et al., 2018) showed how a perfluorinated herbicide (sulfluramid) posed a source of smaller PFAS in vicinal ecosystems on account of its poor biodegradability.

The minor microbial defluorination of PFAS is also likely associated with the short evolutionary timespan available for microorganisms to evolve suitable catabolic strategies (Wackett and McMahon, 2021), especially when comparing with the more favorable dehalogenation outputs of polychlorinated molecules, to which there has been a biogenic selective pressure for millions of years (Mayer-Blackwell et al., 2016). A more direct route for proficient PFAS biodegradation has been linked to strategies of metabolic activation, a series of initial catabolic steps targeting non-fluorinated moieties in PFAS that allow a more facile cleavage of existing C-F bonds (Wackett, 2022). Indeed, defluorination has been shown to preferentially occur when microorganisms attack fluoride-free carbon covalent bonds (e.g., C-S, C-O) of legacy PFAS or arylic carbons adjacent to perfluorinated moieties, as shown during the biodegradation of perfluoroalkyl acids, fluorotelomers or aromatic PFAS by aerobic (*Pseudomonas, Gordonia* or *Bacillus*) (Shaw et al., 2019; Chetverikov et al., 2017; Chen et al., 2015; Feng et al., 2012) and anaerobic bacteria (*Acidimicrobium* or *Dehalobacter*) (Yu et al., 2020; Huang and Jaffé, 2019). Still, efficient catabolic strategies capable of challenging the recalcitrance of PFAS are not widespread among environmental microorganisms, as shown by the generalized lack of catabolic talent to tackle these polyfluorinated structures successfully.

5.3. (Bio)degradability of PFAS in the marine environment

The (bio)degradation of PFAS in the marine environment is still very meagerly studied. As observed in other environmental compartments, PFAS transformation in this environment is more likely to occur abiotically, albeit very slowly (Bolan et al., 2021; Xu et al., 2021). Yet, the ubiquitous distribution of PFAS in the marine environment observed since their widespread circulation (i.e., for about 80 years) is gradually turning into a scenario of legacy pollution, which has shown in the past to have driven the adaptation of environmental microorganisms towards desirable catabolic phenotypes (Wackett and McMahon, 2021; Zanaroli et al., 2015). It is, thus, possible that the decades-long exposure of the marine microbiome to these compounds eventually elicits the emergence of suitable catabolic strategies capable of coping with PFAS pollution in the oceans. Considering that PFAS resist oxidative catabolism and that their defluorination has been preferentially observed in anoxic environments with demanding redox conditions (Yu et al., 2020; Huang and Jaffé, 2019), deep-sea environments where such environmental conditions are usually met and where PFAS tend to accumulate (Smith et al., 2016) may pose as a particular fascinating niches of microorganisms with higher capacity to adapt and respond to the presence of these xenobiotic pollutants.

6. Methods for PFAS analysis and pollution mitigation

6.1. PFAS identification and quantification analytical methods

A wide variety of analytical procedures are available for the determination of PFAS in various matrices (Rehman et al., 2023; Androulakakis et al., 2022; Al Amin et al., 2020a; Gao et al., 2020; Rodriguez et al., 2020). In many cases PFAS analysis is performed by combination of solid phase extraction followed by liquid chromatography coupled with mass spectrometry (LC-MS/MS); this approach is both selective and quantitative with low detection limits in the low ppt range or even lower. In specific cases, gas chromatography (GC) can also be employed, e.g. for the analysis of neutral and volatile PFAS compounds; ionic PFAS compounds can also be determined by GC after derivatization (Gao et al., 2020). As mentioned above, preconcentration of PFAS from water matrices is usually based on the use of appropriate solid phase extraction (SPE) procedure. The standard SPE employs a cartridge containing the sorbent capable of adsorption of target analytes; after washing away undesired components the analytes are eluted with appropriate solvent (Andrade-Eiroa et al., 2016). Solid phase microextraction (SPME) is based on the partitioning of target analytes from the sample to the coating of a small fiber. After extraction for a prescribed time, analytes can be thermally desorbed from the fiber at the GC injector port or eluted with an appropriate solvent for further analysis (Pena-Pereira et al., 2021). Thin film SPE is a combined sampling and sample preparation technique enabling analysis in larger sample volumes employing larger adsorbent surface area (Pena-Pereira et al., 2021). Magnetic solid phase extraction (MSPE) employs magnetically responsive adsorbents that can be selectively, rapidly and efficiently separated from the analyzed samples using appropriate magnetic separators (Pena-Pereira et al., 2021; Safarikova and Safarik, 1999). Also stir bar sorptive extraction (SBSE) employing a magnetic stir bar having three essential parts (magnetic stirring rod, the thin glass jacket and a layer of appropriate adsorbent) can be used for the extraction of various analytes, including extracting PFAS from environmental and biological samples (Yao et al., 2018; Martín et al., 2016).

Fast liquid chromatography techniques including ultra-high performance liquid chromatography (UHPLC) are gradually replacing standard HPLC in PFAS analysis due to the long analysis time and relatively high LOD of HPLC. Columns with smaller particle size and narrower inner diameters in UHPLC enable fast separation and high resolution. The lower solvent and sample consumption of UHPLC in comparison to standard HPLC are also favorable from a green-chemistry and economy perspective (Selahle et al., 2022; Cielecka-Piontek et al., 2013).

Nonetheless, also several other alternative analytical procedures can be successfully employed for PFAS analysis. Direct analysis in real time (DART) mass spectrometry enables the rapid analysis of solid, liquid and gaseous samples at atmospheric pressure without the need for specific sample preparation. During the DART ionization process, analytes can be desorbed at appropriate temperature directly from the studied surfaces (Cody and Maleknia, 2020). Smartphone based image analysis is a progressive low cost procedure employing specific applications for the image analysis of colored samples; usually parameters of several color spaces can be measured. The intensity of sample coloration is usually proportional to the analyte concentration in the tested sample; this process can be used especially as a pre-screening tool with a high level of sensitivity and selectivity (Al Amin et al., 2020b). Typical examples of the above-mentioned solid phase extraction and analytical procedures for PFAS analysis are presented in Table 2.

6.2. Methods for PFAS pollutants extraction and removal

Mitigation approaches for the PFAS pollution are in high demand and must be applied at the pollution sources and not after PFAS reaching the estuarine and marine ecosystems. Factories and industries that produce and use PFAS, as well as water treatment plants, must be

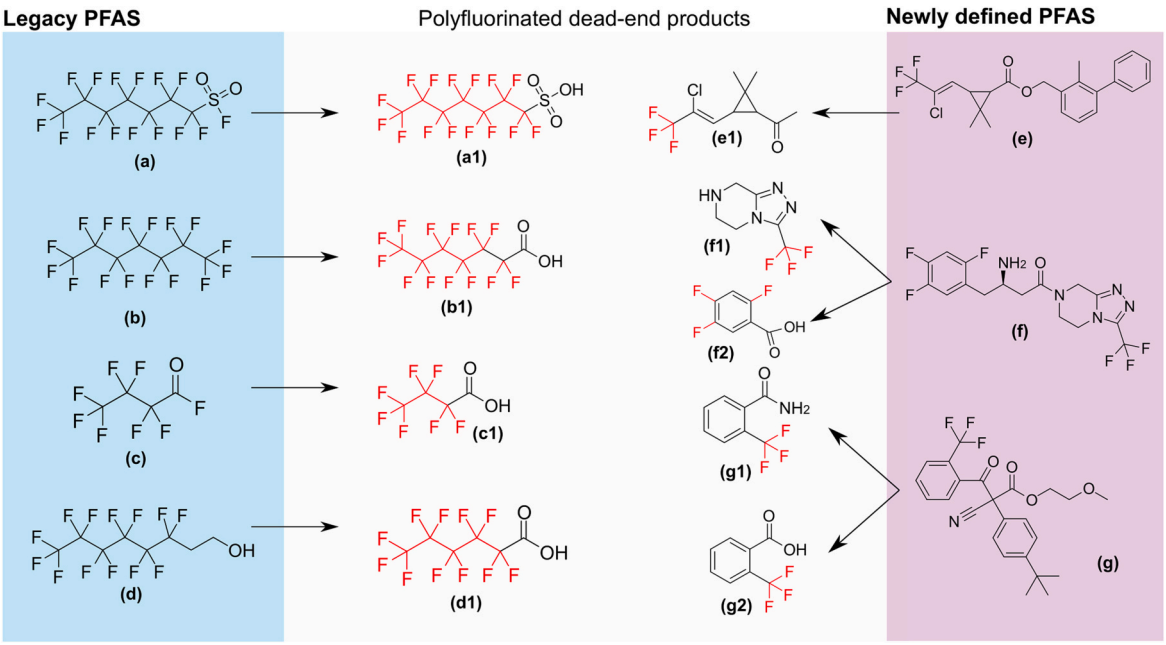


Fig. 2. Chemodiversity of polyfluorinated metabolites stemming from the incomplete biodegradation of some legacy (blue) and newly defined (purple) PFAS. Data was taken from (Li et al., 2020b; Henning et al., 2019; Uniyal et al., 2016; Howard and Muir, 2010; Wang et al., 2009). PFAS nomenclature: (a) 1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoroheptane-1-sulfonyl fluoride; (b) perfluoroheptane; (c) 2,2,3,3,4,4,4-heptafluorobutanoyl fluoride; (d) 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecanofluorooctan-1-ol; (e) bifenthrin; (f) sitagliptin; (g) cyflumetofen; (a1) perfluoroheptanesulfonic acid; (b1) perfluoroheptanoic acid; (c1) perfluorobutanoic acid; (d1) perfluorohexanoic acid; (e1) (Z)-1-(3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropyl)ethenone; (f1) 3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrazine; (f2) 2,4,5-trifluorobenzoic acid; (g1) 2-(trifluoromethyl)benzamide; (g2) 2-(trifluoromethyl)benzoic acid.

accountable for treating PFAS waste.

Currently, there are several PFAS remediation techniques, among the most commonly used and recognized methods for PFAS pollution control are adsorption on natural and renewable material-based adsorbents, agricultural wastes, biochar, and many composite materials (Militao et al., 2021; Omo-Okoro et al., 2018). Other potential procedures for PFAS removal have been summarized in several reviews (Smaili and Ng, 2023; Vu and Wu, 2022; Yadav et al., 2022; Boyer et al., 2021; Gagliano et al., 2020). In addition to PFAS listed as persistent organic pollutants, some new fluorinated organic compounds with similar structures were rapidly available on the market, such as sodium p-perfluorous nonenoxybenzene sulfonate and perfluoro-2,5-dimethyl-3,6-dioxanonanoic acid, also causing water contamination, which needs removal for environmental protection (Wang et al., 2022).

In the near future, adsorption studies, including contaminated water on large scale must be performed. It may be helpful in case of extensive sea contamination due to the use of fire-fighting foams in fire accidents on oil platforms, ports, etc. Table 3 summarizes examples of PFAS removal procedures from contaminated water samples.

The described remediation methods are more effective than microbial biodegradation, bioremediation or potential microbial composting and can be used at industrial scale.

7. The current trends and strategies in PFAS regulation

7.1. *Perfluoroalkoxy substrates market trends and forecast*

The PFAS global market is expected to reach USD 6.8 billion by 2024, driven mainly by increased demand for high-purity PFAS coating in critical fluid transport tubing applications and increased demand for ultra-high pure PFAS resin in the semiconductor industry[3]. The PFAS industry is very disciplinary and is one of the highly significant industrial-added value chains. Thus, in 2018 the downstream application of fluoropolymers, where PFAS are widely used in the United States, was distributed mainly to the sectors of electronics (24 Kt), transportation (19 Kt), chemical and industrial processes (12.5 Kt), consumer products (7.5 Kt), and energy (4 Kt).

The USA industry, which was a net exporter in 2018, had sales of fluoropolymers in their basic form of USD 2 billion and 85 Kt of products, and the sales value of export exceeded USD 1 billion, with imports of USD 500 million. Indirectly, the industry is also estimated to have generated USD 150 million in R&D spillover effects with a further USD 2.4 billion indirect and induced economic activity along with 15,000 direct and indirect USA jobs (The Report Socio-Economic Assessment of the US Fluoropolymer Industry, 2020). In 2020, fluoropolymers had a market value of USD 7.6 billion and are projected to reach USD 10.2 billion by 2027[4]. The market is expected to record a total annual growth rate (CAGR) of 4.3% over the forecasted period. Also, the market volume was around 356.70 Kt in 2020, with an expected CAGR of 3.8%. Globally, the use of fluoropolymers in the automotive industry, construction and infrastructure development is growing steadily, while the aforementioned five sectors cover almost 68% of the fluoropolymers market. Therefore, the fluoropolymers industry and market are accelerating the growth of PFAS production, which Asia dominates with continued growth in the global PFAS market.

The Annex XV Restriction Report[5] by ECHA identified the main uses of PFAS in which the largest amounts of PFAS are used and emitted,

[3] https://www.researchandmarkets.com/reports/4833507/global-perfluoroalkoxy-pfa-market-by-product

[4] https://www.researchandmarkets.com/reports/5411606/global-fluoropolymers-market-by-type-etfe-pfa

[5] https://echa.europa.eu/documents/10162/f605d4b5-7c17-7414-8823-b49b9fd43aea

Table 2
Examples of solid phase extraction and subsequent analytical procedures for PFAS analysis.

Extraction type	Analyzed samples	Extraction system	Elution	Subsequent analysis	Other details	References
SPE	Artificial seawater	Oasis® HLB Strata™-X	Methanol	HPLC-ESI-MS/MS	The influence of type of sorbent, matrix pH, salinity and eluent on the 12 PFAS compounds recovery investigated	(Brumovský et al., 2018)
SPE	Marine water samples from the Saudi Arabian coastal waters of the Red Sea	Waters Oasis® 500 mg HLB cartridges	Methanol	HPLC-MS/MS	The highest PFAS levels have been found in Al-Arbaeen and Al-Shabab lagoons	(Ali et al., 2021)
SPE	Waters from Central and South Florida, USA	Strata-XL AW cartridges	0.3% NH_4OH in methanol	HPLC-MS/MS	PFAS were detected in all tap water (N = 10) and surface water samples (N = 38) with total concentrations up to 169 ng/L	(Li et al., 2022b)
SPE	Rivers and estuaries in Port Philip Bay, Victoria, Australia	Oasis® WAX	0.1% NH_4OH in methanol	HPLC-MS/MS	Examination of the occurrence of common PFAS in waters	(Allinson et al., 2019)
SPE	Water from Pensacola Bay System, Florida, USA (45 different sites)	Strata-X-AW	Methanol followed by 0.3% NH_4OH in methanol	UHPLC-MS/MS	At all sites, at least eight or more PFAS were quantified	(da Silva et al., 2022)
SPE	Tap water and rainwater	Bamboo charcoal	Methanol	HPLC-ESI-MS	LOD for PFOA was 0.2 ng/L. Good linearity ($R^2 = 0.9995$) over the range 1–1000 ng/L observed	(Zhao et al., 2008)
In situ SPE	Spiked water samples	OASIS®WAX	Methanol, ethyl acetate and dichloromethane	GC-MS/MS	An inter-laboratory trial to validate ISO 21675 method for the measurement of PFAS in water samples	(Taniyasu et al., 2022)
Fluoro-SPE	Spiked tap water samples	Fluoro-Gel	Methanol	Smartphone assay	Smartphone-based image analysis due to the coloration of fluorosurfactants with ethyl violet	(Al Amin et al., 2020b)
SPME	Tap water, lake water, bottled water and river water samples	Hydrophilic-lipophilic balance-weak anion-exchange/ polyacrylo-nitrile fibers	Methanol:water (80:20, v:v) adjusted to pH 10 with NH_4OH	UHPLC-MS/MS	This method achieved LOQs up to 1 ng/L with satisfactory precision and accuracy values evaluated over a period of 5 days	(Olomukoro et al., 2021)
SPME	PFAS-contaminated groundwater samples from Northern Queensland, Australia	Octadecylamine coated Pyrex glass capillaries	None	Direct analysis in real time (DART) mass spectrometry	Lowest detection of PFOA at 500 parts-per quadrillion (ppq) in tap water	(Cody and Maleknia, 2020)
Thin film SPME	Seawater from North Myrtle Beach, South Carolina	HLB-WAX/PAN thin films	Methanol:water (80:20) with 2% ammonium formate	UHPLC-MS/MS	The use of thin films resulted in a twofold improvement in extraction efficiency compared to fibers, especially for the short-chain PFAS	(Olomukoro et al., 2023)
MSPE	Drinking, river, snow and pond water samples (China)	Magnetic covalent triazine-based frameworks	Acetone	LC–MS/MS	Six PFAS compounds analyzed. LODs were in the range 0.62–1.39 ng/L	(Ren et al., 2018)
MSPE	Environmental water samples	Cetyltrimethylammonium bromide coated magnetite nanoparticles	Acetonitrile	HPLC-ESI-MS/MS	Seven PFAS compounds analyzed. LODs were in the range 0.022-0.31 ng/L	(Zhao et al., 2011)
SBSE	Environmental water samples	Poly(1-vinylimidazole-ethyleneglycol dimethacrylate) monolith-coated stir bars	Methanol containing 0.4% ammonia (v/v) under sonication	HPLC-ESI-MS/MS	Eleven PFAS compounds analyzed. LODs were in the range of 0.06–0.40 ng/L	(Yao et al., 2018)

ESI – electrospray ion source; LOD – limit of detection; LOQ – limit of quantification; MSPE – magnetic solid phase extraction; SBSE – stir bar sorptive extraction; SPE – solid phase extraction; SPME – solid phase microextraction.

within 14 sectors and/or applications, subdivided in numerous sub-sectors. For the EU, this resulted in an estimated 140 to 310 Kt of PFAS introduced into the market in 2020, which is likely to climb even more under the baseline scenario due to expected economic growth in various industries. The estimated mean PFAS tonnage in the EEA over a 30-year period is 49 000 Kt, resulting in emissions of approximately 4500 Kt during the manufacturing and usage phases if no action is taken. Emissions throughout the waste phase, which may be significant, are not included in this assessment and therefore it can be assumed that the emission estimates are seriously underestimated.

7.2. The main European Union (EU) regulations relating PFAS

Based on a proposal from the Swedish Chemicals Agency (KEMI) and the German Environment Agency (UBA), launched in 2017, the EU decided to ban several PFAS chemicals starting in February 2023[6]. This restriction, also under Regulation (EC) No 1907/2006[7] – Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) is covering six long-chained perfluorinated and polyfluorinated substances (molecules consisting of 9 to 14 fluorinated C atoms), and if any substance can degrade into one of these six substances, it is still valid. In practice, the restriction is expected to apply to approximately 200 PFAS. In recent years, scientists and various authorities have undertaken

[6] https://www.ivl.se/projektwebbar/baltic-sea-pfas-network/pfas-information.html

[7] https://www.informea.org/en/legislation/regulation-ec-no-19072006-european-parliament-and-council-concerning-registration

Table 3
Examples of adsorbents for PFAS compounds removal from contaminated water samples.

Polluted samples	Adsorbents	Experimental details	References
PFAS contaminated water samples	Biosolids derived biochar	Biochar demonstrated >80% adsorption of long-chain PFAS and 19–27% adsorption of short-chain PFAS	(Kundu et al., 2021)
Contaminated water from a well in a factory producing water resistant clothing	Coal derived activated carbon	Activated carbon was efficient PFAS adsorbent. The Freundlich adsorption model followed.	(Hansen et al., 2010)
Contaminated groundwater	Surface modified organoclay, granular activated carbon and ion exchange resin	Ion exchange resin exhibited the best PFAS adsorption (nearly four times higher than activated carbon and two times higher than organoclay)	(Murray et al., 2023)
PFAS-contaminated groundwater samples from fire training areas, burn pits, and other water sources	Metal−organic frameworks	PFAS adsorption was dominated by electrostatic and acid−base interactions for anionic and non-ionic PFAS, respectively; preferred for long- over short-chain PFAS; strongly dependent on the nature of PFAS head group functionality	(Li et al., 2021a)
PFAS-contaminated groundwater samples from U.S. Air Force bases	Zirconium-based metal−organic framework	PFAS removal rates of 75−98% within 10 min regardless of the presence of co-contaminants	(Li et al., 2021b)
PFAS-contaminated water from the Xiaoqing River basin	β-Lactoglobulin amyloid fibril membrane	Membrane exhibits high efficiency for removing both high (>μg/L) and trace (ng/L) levels of the PFAS compounds	(Jin et al., 2021)
Aqueous film-forming foams wastewater from a fire-fighting manufacturing company containing PFAS	Magnetic fluorinated vermiculite	Adsorbent exhibited very fast and selective adsorption of perfluorooctane sulphonate in the presence of other compounds. Regeneration with methanol.	(Du et al., 2017)

campaigns to draw attention to the negative repercussions of the widespread use of PFAS in industry and daily life consumables. At the EU level[8], PFOS have been restricted under the EU persistent organic pollutants (POPs) Regulation EU, 2019/1012[9]) Regulation EU 2019/1021 for more than ten years; while PFOA and PFOS are listed in Stockholm Convention[10] (including 152 signatory countries) as substances that should be eliminated/banned in production and used as chemicals. For more details see section SI2: Supplementary Information on PFAS Regulations.

Furthermore, PFAS policy is becoming one of the priorities in the EU Chemicals Strategy for Sustainability (CSS) presented on 14 October 2020, which highlights the priority to eliminate endocrine disruptions caused by persistent and highly mobile chemicals, such as PFAS. Efforts to overcome significant barriers to effectively identifying and regulating the most dangerous chemicals seem crucial, as actual data on their presence is often very insufficient. While the REACH Regulation, driven by the motto *no data, no market*, requires industry to manage chemical hazards and publish safety information on compounds, companies in transition and developing countries operate unhindered in a *no data, no problem* manner instead of being penalized for it.

7.3. Efforts and good practices aimed at reducing the impact of PFAS in marine environment

7.3.1. Policy Area (PA) hazards - EU Strategy for the Baltic Sea Region (EUSBSR)

The European Commission launched the EU Strategy for the Baltic Sea Region (EUSBSR) in 2009, which provided a framework for Baltic Sea-bordering Member States to collaborate on three broad objectives: i) to protect the sea; ii) increase prosperity; and iii) connect the region. Moreover, in the current revision of EUSBSR[11], the main objectives were not significantly modified. EUSBSR Policy Area Hazards (PA Hazards) was coordinated by Sweden through the Sweden Environmental Protection Agency and guided by an international Steering Group comprised of national experts from relevant public bodies from all countries around the Baltic. Within the EUSBSR environmental objective "*Save the Sea*" and several sub-objectives such as "*clear water in the sea*", "*rich and healthy wildlife*" and "*better cooperation*", PA Hazards strived to reduce the use and impacts of hazardous substances.

In 2017, PA Hazards commissioned the study"*PFAS in the Baltic Sea Region: Inventory of awareness, actions and strategies related to highly fluorinated substances, PFAS, including PFOS*" (Sahlin, 2017). This study was performed based on a questionnaire, which inventoried national strategy and data on firefighting foams, groundwater and drinking water, and contaminated soil in Sweden, Denmark, Germany, Finland, Poland, Lithuania, Latvia and Estonia. Based on the study results, in 2018, PA Hazards International Steering Group considered the need for further work on PFAS in the Baltic Sea region and launched the Baltic Leadership Programme on PFAS ("PFASeout")[12] intending to raise knowledge among policy actors. The medium-term objective of the programme (2020-2030) is to improve PFAS data collection, regulation and public-private sector collaboration on their use in products and release to the environment; the long-term objective (2025+) of the programme is to significantly decrease the environmental release of PFAS into the Baltic Sea catchment area. The programme has the PFAS Reference Group (PFAS RG), which consists of the Swedish Environmental Protection Agency, the Swedish Chemicals Agency, the Swedish Agency for Marine and Water Management, the Finish Environment Institute, the Swedish Institute and the Baltic Marine Environment Protection Commission HELCOM[13]. Currently, the Baltic Sea PFAS Network[14] connects stakeholders, whose main goal is reducing environmental pollution with PFAS. This platform also ensures knowledge sharing, experience transfer and networking for opportunities in PFAS usage and management, removal, environment impact, policy and regulations.

[8] https://echa.europa.eu/hot-topics/perfluoroalkyl-chemicals-pfas
[9] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R1021
[10] http://chm.pops.int/Countries/StatusofRatifications/PartiesandSignatoires/tabid/4500/Default.aspx
[11] https://www.eusbsr.eu/
[12] https://klaipedaregion.lt/wp-content/uploads/2019/07/Baltic-Leadership-Programme-on-PFAS_Concept-for-Participants.pdf
[13] https://helcom.fi/
[14] https://www.ivl.se/english/ivl/project/baltic-sea-pfas-network.html

7.3.2. An example of good practice in reducing PFAS production – contribution to EUSBSR

The Interreg project NonHazCity[15] (2016-2019) enabled nine municipalities across the Baltic Sea to develop their chemical action plans for hazardous substances entering the Baltic Sea and trained dozens of small businesses and households to reduce their emissions. The project results showed that companies are generally not concerned about problems with PFAS just because they are not aware that they are using hazardous substances. Although dangerous chemicals management is complex, simple and convincing strategies to improve hazardous material awareness among enterprises are needed. There are many ways in which municipalities can motivate companies to reduce the use of hazardous substances: raising awareness, advising on the replacement of hazardous substances in the workplace, engaging with professional associations and trade organizations, as well as better considering the hazardous substances in the procurement process. The project clarified that municipalities, in particular, could help businesses to play a much better role than they currently do in reducing hazardous substances in homes, workplaces and the environment; they could also take the lead in facilitating collaboration between companies and raising awareness of the chemicals present in products and materials, and significantly reduce the impact of PFAS on human health and the local/regional environment (NOnHazCity Project Report, 2019).

7.3.3. An example of good practice in reducing PFAS production – contribution to activities of the US Enviromental Protection Agency (EPA)

Although there appear to be measured in place to monitor and reduce PFAS pollution, there should be a clear understanding that meaningful action is required not only to address remediation and clean-up of inherited contamination but also to mitigate current PFAS production and use to limit the scope of future exposures (Cordner et al., 2021). Thus, in the United States, eight major PFAS manufacturers voluntarily joined the Environmental Protection Agency's (EPA)[16] PFOA Stewardship Program in 2006, committing to continuously invest more than 700 million USD in R&D, with the commitment to discontinue the production and use of PFOS and PFOA-related chemicals and the agreement to subject the new PFAS chemistry to enhanced regulatory revision before it is allowed on the market. The FluoroCouncil[17] was established as part of the 2010/2015 PFOA Stewardship Program to i) maintain the transition from older fluorinated chemistries, commonly referred to as long-chain PFAS, to newer products that include short-chain PFAS, which, according to manufacturers, have significantly improved health and safety profiles; and ii) examine the science and society that addresses questions about the safety profile of these substances.

7.4. Importance and multiple benefits of monitoring

The World Health Organization (WHO) consistently warns that the monitoring and regulation related to PFAS are crucial and should be implemented not only at the national and regional level but also at the global level. The emergence of PFAS in water, its persistence in the environment and bioaccumulation in living organisms is becoming an increasingly widespread problem. According to a recent review by Baluyot et al. (Baluyot et al., 2021), temporal monitoring sampling strategies that provide trends and status of PFAS in the environment and population are already in place in the USA and the EU, while awareness of PFAS exposure in Asia is still in its infancy. In comparison to the USA, PFAS levels in South and Southeast Asia were just below the recommended level. Still, the rise of PFAS in China in the last decade suggests that increased PFAS contamination in South and Southeast Asia may follow soon as these countries compete in the global economy (Baluyot et al., 2021).

7.4.1. The case study "PFASs in the Nordic Environment"

Nordic initiatives and studies on PFAS cover a wider area than just the Baltic Sea region. In 2019, two studies were launched at the Nordic Council of Ministers. The monitoring study "*PFASs in the Nordic environment. Screening of Poly- and Perfluoroalkyl Substances (PFASs) and Extractable Organic Fluorine (EOF) in the Nordic Environment*" (Kärrman et al., 2019) was initiated in 2019, based on the results from 2004, when the Nordic region first started screening for PFOS, PFOA, PFHxA, perfluoroheptanoic acid (PFHpA), PFNA, PFHxS, perfluorodecanoic acid (PFDS) and perfluorooctane sulfonamide (PFOSA) in surface water, seawater, sediments, wastewater, sludge, fish, shellfish, molluscs, crustaceans (marine and freshwater species), marine mammals and seabirds; in which PFOS and PFOA were found in almost all samples. The rapid progress of analytical tools and quantification methods has increased the detection limit of PFAS and their conjugates over the last two decades. The aim of this study, conducted on behalf of the Nordic Screening Group, was to monitor for the first time an extensive list of legacy and newly PFAS in a wide range of ecological matrices from the Nordic countries and to compare the results with measured total extractable organic fluorine, to encompass all unknown fluoroorganic compounds. A total of 99 substances were analyzed, divided into the following categories: i) volatile PFAS (vPFAS); ii) ultra-short chain PFAS; iii) perfluoroalkyl carboxylic acids and sulfonic acids (PFCAs and PFSAs); iv) precursor PFASs; v) perfluoroalkyl phosphonic and phosphinic acids (PFPA/PFPiAs); and vi) novel PFAS. A total of 102 samples were analyzed, including bird eggs, fish, marine mammals, terrestrial mammals, surface water, WWTP effluents and sludge, and air. Samples were collected by institutes from the participating countries and self-governing areas: Denmark, Faroe Islands, Finland, Greenland, Iceland, Norway, and Sweden, with most of the samples collected since 2017. The results of the study showed the need to include more classes of PFAS in environmental assessments. Short-chain PFAS with a C2-C4 chain length, frequently detected in surface water and WWTP effluents, are likely to be as persistent as their long-chain homologues, while their long-term effects on the environment and humans are still unknown. Precursor compounds were also frequently detected in many samples. The large proportion of unknown extractable fluoroorganics in most environmental samples in the Nordic environment also requires further studies and warranted monitoring.

7.4.2. The case study: "The Cost of Inaction"

Another study launched at the Nordic Council of Ministers was dedicated to the socio-economic aspects of the impact of PFAS - "*The cost of inaction: A socio-economic analysis of environmental and health impacts linked to exposure to PFAS*" (Goldenman et al., 2019) with the explicit aim of raising awareness of the long-term effects of PFAS on the environment and human health, taking into account only socio-economic costs. The scope of this study concerned only C4-14 non-polymeric fluoro-surfactants in the European Economic Area (EEA). According to the report, PFAS contamination will remain on the planet for hundreds, or even thousands of years, human and environmental exposure will continue, and exposure mitigation efforts will lead to high socio-economic costs that will be borne mainly by governments and taxpayers. The analyzed case studies showed that a large part of PFAS is released into the environment as a consequence of their production, use in the production of other products and through the use of products containing PFAS. According to the study, up to 20 facilities are currently producing fluorochemicals in Europe, including the Nordic region. These facilities are considered the main source of PFAS released into the environment, with worker exposure also being high. The costs of remediation, which often does not result in the complete removal of PFAS, in some cases range up to several million EUR, while the total costs at the European level can reach hundreds of millions of EUR.

[15] https://projects.interreg-baltic.eu/projects/nonhazcity-7.html
[16] https://www.epa.gov/
[17] https://fluorocouncil.com/

However, disclosed results on health-related costs to society and related to environmental contamination highlighted the heavy costs that society will have to pay in the future if actions are not taken. Thus, the estimated annual health-related costs range due to PFAS exposure is 2.8 – 4.6 billion EUR for the five Nordic countries and 52 – 84 billion EUR for all EEA countries. The actual prices are likely higher, as these calculations refer to only a few of the health effects associated with PFAS exposure. The total range of estimated environmental costs is 46 million –11 billion EUR over the next 20 years for the Nordic countries, and for all 31 EEA Member States and Switzerland, the range of costs for environmental remediation is 821 million –170 billion EUR. Although this study does not directly address the impact on the marine environment, it does quantify the costs associated with the loss of ecosystem services. In this sense, on all four categories of coastal and marine ecosystem services, such as support (e.g. nutrient cycling), supply (fishing, seafood production), regulation (carbon sequestration in the ocean), as well as coastal and marine cultural services (tourism, recreational, aesthetic and spiritual benefits) the "cost of inactivity" can be assessed.

8. The legal framework for PFAS pollution control in marine management

The EU Committees for Risk Assessment (RAC) and Socio-Economic Analysis (SEAC) on their meeting in December 2021[18], have supported Germany's proposal to restrict the use of undecafluorohexanoic acid (11-PFHxA), its salts, and related substances, according to the European Chemicals Agency (ECHA)[19], responsible for implementing EU chemical regulations. 11-PFHxAs, a subgroup of PFAS, are widely used in many sectors (e. g., paper and cardboard packaging, textiles, and fire-fighting foams) and are known as very persistent and mobile compounds. It is anticipated that short-chain perfluorinated compounds such as PFHxA (C6) are intended to replace long-chain (C8 to C14) perfluorinated substances (e.g. PFOA, PFCA) because many of these harmful substances are or will soon be prohibited (e.g. PFOA, PFCAs) due to their toxicity. Although this support seems very promising, the reasons offered for limiting the use of these substances to reduce further environmental and human exposure do not even mention the marine ecosystems or marine organisms.

Furthermore, the International Convention for the Prevention of Pollution from Ships (MARPOL)[20] is the primary international convention that regulates pollution from ships and other vessels, intending to prevent and/or minimize the pollution (operational or accidental) of the marine environment. In the six MARPOL annexes covering the pollution caused by several substances, including oil, noxious liquid substances, sewage, garbage and air pollution, PFAS are not mentioned. The potential threat of PFAS to the marine environment was probably not recognized at the time of the adoption of the MARPOL Convention in November 1973. However, today it seems surprising that PFAS are still not listed as hazardous substances, especially given their lasting and recalcitrant effects on the marine environment and its biodiversity (Strodder, 2020).

The Descriptor 8 of the Marine Strategy Framework Directive (MSFD) (Directive 2008/56/EC) regulates the *protection of the pollution of marine water by chemical contaminants* and is closely related to the Water Framework Directive (WFD) 2000/60/EU[21] (Directive 2000/60/EC), which covers freshwaters and some marine waters. According to the MSFD, monitoring is required to conduct an integrative assessment of the environmental state, with the scopes of the WFD and MSFD overlapping in terms of coastal waters (Fliedner et al., 2020). In contrast, recommended substances for monitoring in marine environments are initially selected under the WFD 2000/60/EU and 2013/39/EU[22] (Directive 2013/39/EU). In this regard, PFOA and PFOS are considered priority hazardous substances under the WFD 2000/60/EU, while PFOS and their derivatives are included as a priority hazardous substance under the Directive 2013/39/EU with a much lower Environmental Quality Standard (*anual average*; AA-EQS) limit value of 0.65 ng/L in inland surface waters and 0.13 ng/L in seawater. Thus, samples taken in 2013 in Northern Europe exceeded this EQS in 27 % of river sites and 94 % of Baltic Sea and Kattegat seawater (Nguyen et al., 2017). Ten years later, a comprehensive report on spatio-temporal trends, bioaccumulation and compliance with the new EQS for PFAS has been published by the Swedish Marine Contaminant Monitoring Programme (Soerensen and Faxneld, 2023). PFAS analyzes were conducted on four fish species and three bird species, including 40 years of the longest time series monitoring and 26 monitoring stations, from the Bothnian Bay in the north to the Skagerrak on the west coast of Sweden (called the Great Baltic Sea). After the initial exponential increase in PFAS concentrations, there was a rapid response to the introduction of stricter regulations in the early 2000s, which resulted in the stabilization of PFAS concentrations in the marine environment. With the exception of PFOA (C8) and PFNA (C9), which continue to show significant increasing trends at selected monitoring stations, PFAS concentrations have considerably declined at most monitoring stations over the last ten years (especially PFSA and PFCA with chain lengths longer than C9). Unfortunately, PFAS concentrations in biota are still 5-230 times higher than the threshold recommended in the PFAS EQS dossier (Soerensen and Faxneld, 2023).

Specifically, Australia and New Zealand developed the PFAS National Environmental Management Plan (NEMP)[23], which provides nationally agreed guidance on the management of PFAS contamination focused on ambient environmental monitoring to establish baseline data and identify temporal and spatial trends in the concentration and presence of specific PFAS. Fresh and marine surface waters, sediments (freshwater, estuarine, and coastal), biota (e.g. marine tissue samples), groundwater, air, and soil are among the environmental aspects that should be considered for inclusion in the environmental monitoring program. It is encouraging that despite each state and territory having its own legislation, NEMP shows how states and territories have a unified, unambiguous and consistent approach to PFAS environmental protection (HEPA, 2020).

8.1. The future challenges and recommendations for marine and coastal management

PFAS spills typically occur when the fire-fighting foam is used on tankers and oil rigs after accidents and reaches the marine environment. The most important legal issues related to PFAS as "forever chemicals" are their long-term danger and the possibility of future lawsuits. This means that the maritime industry and related companies whose employees have been exposed to PFAS may face expensive and unexpected claims in the future. Accordingly, the maritime industry must manage the potential of future long-term responsibility by adopting clear policies, procedures and plans on how to use, store, handle and dispose of PFAS following relevant national environmental regulatory legislation. Industries need to be well acquainted with regulations and ethics, Responsible Research, Innovation and Development concept, and complying with the UN Sustainable Development Goal 14, (UNSDG14)

[18] https://www.lisam.com/en-us/lisam/news/rac-and-seac-support-germany-s-proposal-to-restrict-the-pfas/

[19] https://echa.europa.eu/

[20] https://www.imo.org/en/About/Conventions/Pages/International-Convention-for-the-Prevention-of-Pollution-from-Ships-(MARPOL).aspx

[21] https://www.eea.europa.eu/policy-documents/water-framework-directive-wfd-2000

[22] https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:en:PDF

[23] https://www.dcceew.gov.au/sites/default/files/documents/pfas-nemp-2.pdf

"Life below water". This goal aims to conserve, sustainably manage, and protect marine and coastal ecosystems to avoid significant adverse impacts due to human actions, reinforcing ecosystems strength, and act for their restoration to achieve healthy and productive oceans for the sustainable use of its resources. In fact, the EU aims to protect and monitor 30% of its seas by 2030, through the creation of marine protected areas (MPAs) and has declared zero-tolerance towards illegal practices and nonsustainable harvesting of marine resources (Schneider et al., 2022). In this context, Responsible Research and Innovation (RRI) enable us to meet societal needs considering ethics and sustainability, being the use of PFAS no exception to these premises (Schneider et al., 2022, 2023).

In an era of rapid global biodiversity loss, a carefully planned marine/coastal management program with new monitoring tools can mitigate the pollution damage already done to marine living organisms. Social responsibility is highly essential in industrial production, which can be achieved through the development and implementation of technological advances, process improvements and the design of production methods that increase productivity and reduce waste. Marine ecosystems and their dwellers have long faced the consequences of decades-long negligence and failure to take measures to reduce pollution. In addition, the challenges of sustainable waste management are particularly pronounced in developing and transition countries, mainly due to inefficient local management systems and low interest in governing structures in this issue. Indeed, current trends in marine/coastal management require an interdisciplinary approach followed by local and regional management planning, stable financial mechanisms and instruments to support the sustainable development of these environments. In the long run, the best solutions will be achieved by forming international consortia capable of implementing large-scale projects whose results will improve the protection of the marine environment and the preservation of certain regional seas, with a focus on marine protected areas. Moreover, these activities include improving existing capacities and, in particular, capacity building within transition countries to meet current trends in marine litter management, with the aim of biodiversity conservation.

Therefore, it is generally accepted that the social dimensions of resource use, with strong local community involvement, should be supported by the government in improving management efficiency. Consequently, the focus should be on locally managed marine areas (LMMAs) with a vital role for the local community, whenever possible, which is particularly important in protecting the coastal regions and marine animals, such as sea turtles, and seabirds, among others.

As highlighted in this review, further efforts to mitigate marine environmental pollution caused by PFAS are imperative and should consider the following aspects:

1. Determining primary sources of legacy and newly defined PFAS contamination in the marine ecosystems and their main transport routes (based on spatial data);
2. Increasing monitoring activities for MPAs and Marine Natura 2000 sites as a valid addition to the marine mammals protection plan (MMPP) and a base for decisions by governmental bodies/political planners responsible for environmental protection management;
3. Establishing standardized methods for sampling, analysis and reporting data, which could improve and advance research on PFAS monitoring and risk assessment;
4. Investigating the biota and their role in the trophic transfer and PFAS bioaccumulation through the food web, as well as PFAS interactions with MPs, with a focus on i) benthic organisms, which are generally exposed to higher PFAS concentrations due to their closer proximity to sediments; and ii) seabirds and the longest-lived marine mammals that accumulate higher concentrations in their tissues such as liver and endocrine glands, which should therefore be subject to higher protection;
5. Developing PFAS remediation technologies and fomenting their implementation in municipal and State practice for wastewater and leachate treatment, PFAS production facilities, and promoting sustainable investment policies to reach these targets;
6. Improve marine and coastal management in MPAs, particularly in vulnerable marine ecosystems such as marine and anchialine caves or habitats that are difficult to explore (e.g. deep sea). In the marine coastal areas, the focus should be on LMMAs with a key role for the local community in the protecting endangered sea turtles and seabirds.

9. Conclusions

Ocean pollution is a growing global issue that has a plethora of harmful repercussions for marine environments, wildlife and, ultimately, human health. The problem of legacy and newly defined PFAS in the environment goes beyond identifying contamination sources and emphasizing the harmful effects on living organisms and their habitats. It is critical to contextualize the problematic of PFAS marine pollution in a larger framework, considering the synergistic effects of PFAS with other marine pollutants, particularly MPs and heavy metals, and understand how that potentially impacts the abundance of ecosystems services the oceans provide.

Moreover, public health consideration often overlooks the critical importance of the ocean, whose health is becoming closely intertwined with our own, as advocated by the One Health concept. Regrettably, the same chemical properties that contribute to PFAS' commercial attractiveness are also responsible for their environmental persistence and, consequently, for long-term adverse impacts on living beings. Due to their behavior in the environment, PFAS pose a threat mainly to wildlife at higher trophic levels, by biomagnification, consequently affecting protein-rich tissues such as liver and blood. It is expected to be a serious threat to global biodiversity, habitat loss, oceans and human health in the years to come.

Water bodies, especially the ocean, are considered the ultimate destination for PFAS and MP. Particular attention is drawn to the growing concern about the synergistic effects of PFAS with other pollutants, including MP and heavy metals. Very worrisome is the fact that organisms consuming PFAS-sorbed MPs may result in and increased combination of toxic effects than when exposed to these pollutants alone. A new matter of concern is the possibility of MPs particles acting as vectors for PFAS transmission and faster expression of harmful effects on susceptible tissues, especially liver and blood. The possibility of passage the blood-brain barrier (BBB) is accompanied by increased neurotoxicity, changes in behavior and communication, which particularly endangers marine mammals. We can assume that the numerous already known harmful effects of MPs and NPs will be even more expressed in synergy with PFAS and other persistent pollutants in the marine environment, whereby all ocean dwellers may be exposed to such scenario. As the capacity of marine ecosystems to cope with current PFAS pollution levels is unknown, mitigation approaches should especially focus on the prevention of their influx to the oceans, essentially acting on the major sources of emission of these compounds, like industries and wastewater treatment plants. In addition, the updated definition of PFAS has welcomed a new layer of complexity to the already complex context of PFAS-related environmental management and regulation, which should be met with courageous regulatory efforts and unconventional management efforts.

Overall, this review brings together data from several disciplines in an effort to fill existing knowledge gaps in the field, facilitating researchers' selection of experimental techniques and approaches in the search for answers to the problem of PFAS contamination.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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