Chapter 12
Nanoplastics in the Aquatic Environment. Critical Review

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Abstract A growing body of literature reports on the abundance and effects of plastic debris, with an increasing focus on microplastic particles smaller than 5 mm. It has often been suggested that plastic particles in the <100 nm size range as defined earlier for nanomaterials (here referred to as 'nanoplastics'), may be emitted to or formed in the aquatic environment. Nanoplastics is probably the least known area of marine litter but potentially also the most hazardous. This paper provides the first review on sources, effects and hazards of nanoplastics. Detection methods are in an early stage of development and to date no nanoplastics have actually been detected in natural aquatic systems. Various sources of nanoplastics have been suggested such as release from products or nanofragmentation of larger particles. Nanoplastic fate studies for rivers show an important role for sedimentation of heteroaggregates, similar to that for non-polymer nanomaterials. Some prognostic effect studies have been performed but effect thresholds seem higher than nanoplastic concentrations expected in the environment. The high surface area of nanoplastics may imply that toxic chemicals are retained by nanoplastics, possibly increasing overall hazard. Release of non-polymer nanomaterial additives from small product fragments may add to the hazard of nanoplastics. Because

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of the presence of such co-contaminants, effect studies with nanoplastics pose some specific practical challenges. We conclude that hazards of nanoplastics are plausible yet unclear, which calls for a thorough evaluation of nanoplastic sources, fate and effects.

12.1 Introduction

Today, pollution with plastic debris and plastic fragments has been recognized as a major water quality problem in fresh and marine water systems. Various recent reviews address the sources, abundance and negative effects of plastic litter (e.g. Derraik 2002; Andrady 2011; Hammer et al. 2012; Koelmans et al. 2014a), including several other chapters in this volume (Browne 2015; Galgani et al. 2015; Thompson 2015). Science in this field is evolving rapidly, with initial studies mainly focusing on detection and abundance of >5 mm macroplastic in marine ecosystems and biota, followed by an increasing focus on <5 mm microplastics ranging down to the μm-scale. Implications of nanometre-sized plastic particles ('nanoplastics'), constitute a very recent area of the environmental sciences. Nanoplastics are of specific interest because of their nano-specific properties, which fundamentally differ from those of the same polymer type in bulk form (Klaine et al. 2012). A clear definition of what should be named a 'nanoplastic' has not yet been provided. For the sake of this review we suggest to follow the definition used for non-polymer nanomaterials, implying that a plastic particle is said to be nano-sized if it is <100 nm in at least one of its dimensions (Klaine et al. 2012). This links the name of the size class to the most convenient scale to actually express this size (i.e. nanometre), it assures a focus on the nano-specific properties and thus their associated hazards, it avoids confusion with the broad scientific field of nano-EHS, and it ensures that a discussion of regulatory implications of nanoplastics may benefit from the past and present developments in the regulation of other manufactured nanomaterials. It must be noted that the classification of plastic particles is not a trivial issue. Earlier, microplastic has been defined as all particles <5 mm, thus automatically including nanometre-sized plastic particles (Arthur et al. 2009). Another recent definition uses <20 μm as a criterion to classify nanoplastics (Wagner et al. 2014), similar to the cut off used by plankton ecologists for nanoplankton. This definition thus includes micrometresized particles. Furthermore, it must be stressed that in the fields of nanotechnology and material science the term 'nanoplastics' is already used for those plastics that have nanoscale additives to give the material specific properties (e.g. Bussière et al. 2013). In this chapter on environmental implications, we classify nanoplastic (NP) as particles <100 nm for the reasons stated.

NPs is probably the least known area of marine litter but potentially also the most hazardous. Various sources of NPs have been suggested such as release from products or formation from larger particles ('nanofragmentation') (Andrady 2011; Shim et al. 2014; Cózar et al. 2014). Detection methods are in an early stage of

development but to date no NPs have been detected in natural aquatic systems. Some first prognostic bioaccumulation and effect studies have been performed (Brown et al. 2001; Ward and Kach 2009; Bhattacharya et al. 2010; Wegner et al. 2012; Lee et al. 2013; Casado et al. 2013; Besseling et al. 2014b) but there is no systematic effect assessment for relevant aquatic species let alone for the community or ecosystem level. Apart from physiological consequences, NPs might also have chemical effects. The high surface area of NPs may cause exceptionally strong sorption affinities for toxic compounds (Velzeboer et al. 2014a), potentially leading to cumulative particle and chemical toxicity effects once NPs have passed cell membranes. Furthermore, if nanofragmentation is a relevant process, release of non-polymer nanoscale additives from the product fragments may further add to the overall hazard (Nowack et al. 2012).

The aim of this chapter is to present and critically discuss the literature on detection, sources, fate and effects of NPs. Because the literature on NPs is still limited, our synthesis builds on knowledge about bulk polymers i.e. micro- and macroplastics as well as on knowledge about non-polymer nanomaterials. Challenges in performing ecotoxicity tests with NPs are discussed and an outlook to future work and recommendations are provided. The potential effects of NP on human health are covered by Galloway (2015).

12.2 Sources, Detection and Occurrence of Nanoplastic

12.2.1 Sources of Nanoplastic

Primary sources of NPs may relate to release from products and applications, in which nanoplastics are used or formed and that result in emissions to the environment during the product life cycle. Product categories may include waterborne paints, adhesives, coatings, redispersible lattices, biomedical products, drug delivery, medical diagnostics, electronics, magnetics and optoelectronics. Recently, thermal cutting of polystyrene foam has been shown to emit nanometre-sized polymer particles, in the range of ~22–220 nm (Zhang et al. 2012). Many polymers undergo similar thermal treatments during their life cycle. 3-D printing has been shown to emit nanometre-sized polymer particles, in the range of ~11–116 nm, at considerable rates (Stephens et al. 2013). Polystyrene and polyethylene nanoparticles are easy to synthesize (e.g. Lu et al. 2009; Rao and Geckeler 2011), are used for research and other applications and thus will find their way into the environment. Several medical applications include polymeric nanoparticles, nanospheres and nanocapsules, used for drug delivery (Guterres et al. 2007), which are, however, biodegradable solid lipids. Although formally within scope, we argue that such nanoplastics are not likely to be hazardous because of their low persistence in the environment. Cosmetic products are often mentioned in the context of nanoplastics. However, recent product inventories show lowest sizes of ~4 μm present in exfoliating scrubs or skin cleansers (Fendall and
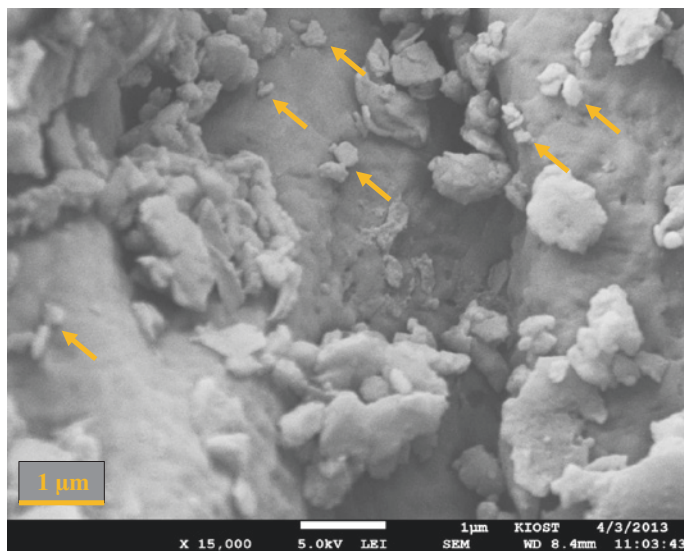
Fig. 12.1 Scanning electron microscopy image of micro- and nano-sized polystyrene particles attached on surface of polystyrene spherule, which were fragmented from the expanded polystyrene spherules by accelerated mechanical abrasion (tumbling at 113 rpm in a glass bottle) with glass beads (3 mm in diameter) for a month. Nanometre-sized particles are indicated by *yellow arrows*

Sewell 2009), rendering these products as an unlikely primary source of NPs. A second speculated source is fragmentation of microplastic to smaller-sized particles eventually reaching the nanoscale (Andrady 2011). Electrospinning of engineered plastics is used to produce mats with nanoscale fibres, which when applied in products might degrade further to the nanoscale (Lu et al. 2008). Polymers consist of a mixture of polymer chains of various lengths. The chains are chemically linked by weak secondary bonds (i.e. hydrogen or Van der Waals bonding) or by physical interaction through entanglement of chains, whereas there is void space in between the chains. The weak interactions are susceptible to breakage at a low energy level. This breakage brings embrittlement, which in combination with other external forces such as friction may cause formation of small particles in the nano-, micro- and millimetre size range, at the surface of the plastics. Shim et al. (2014) were the first to actually report fragmentation of expanded polystyrene (EPS) beads to micro- and nano-sized EPS in experiments involving a month of accelerated mechanical abrasion with glass beads and sand. Formation of nanometre-sized EPS was confirmed with scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) (Fig. 12.1). Without yet even taking UV exposure into account, these experimental conditions may already mimic conditions at beaches or river banks where prolonged abrasion of macro- and microplastics by sand particles possibly leads to the formation of NPs. The combination of photo-oxidation by UV exposure, high temperature and high humidity at beaches probably enhances fragmentation rates and reduces the size of the plastic

particles. However, the occurrence and relative importance of this process still has to be validated in the field. Still, given the available information, we suspect that physical abrasion is a relevant source of NPs.

Although not proven, degradation of microplastics down to the <100 nanometre-scale may constitute a third source of NPs. Slow weathering by photodegradation is well known for all kinds of polymers (Sivan 2011), which is the reason that nano-composites use manufactured nano-particles (nanofillers) to increase the resistance to oxidation (e.g. Grigoriadou et al. 2011; Bussière et al. 2013). UV-B irradiation aided photo-oxidation of LDPE has been shown to lead to the formation of extractable oxygenated compounds as well as non-oxidised low-molecular weight hydrocarbons, which were utilized by bacteria leading to an LDPE mass loss of 8.4 % in 14 days (Roy et al. 2008). The LDPE films subsequently were too fragile to handle. In a recent environmental study, degradation of 1–1.75 % of PE mass was observed in the laboratory in 30 days, by micro-organisms isolated from marine waters present at high densities (Harshvardhan and Jha 2013). Koelmans (2015) suggested that a surface degradation based particle shrinking model may be applied to assess the time dependence of the loss of plastic volume. Using this model and laboratory volume loss rate data from Harshvardhan and Jha (2013), we calculated the time scales required to reach the 100 nm nanoscale as a function of initial plastic particle size. It appears that if oxidation/degradation of the plastic surface would be the rate-limiting process, the rather optimal conditions in the laboratory still would predict that ca. 320 years are needed to bring 1 mm (1000 μm) microplastics to the 100 nm nanoscale (Fig. 12.2). In the oceans, degradation can be assumed to proceed
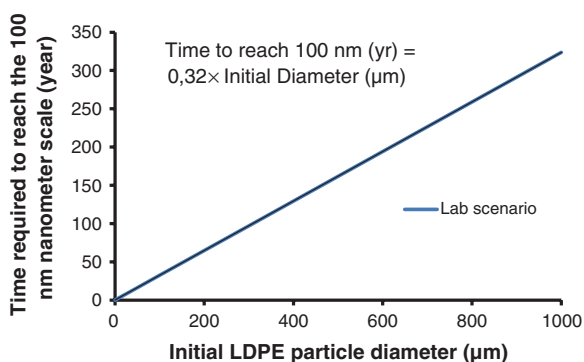

Fig. 12.2 Time required to reach the nanoscale (100 nm) by joint photo-oxidation and biodegradation at the polymer surface, as a function of initial microplastic particle size. The scenario calculation assumes particle shrinking due to photo-oxidation and biodegradation only, and neglects embrittlement and erosion. The reaction rate is proportional to the surface area with rate constant, k' as in $dV(t) = -k'A(t)dt$ with V (m^3), and A (m^2) are particle volume and surface area, respectively. The 'Lab scenario' is based on a mass loss of ~1 % low density polyethylene (LDPE) per month as observed under laboratory conditions by Harshvardhan and Jha (2013). It appears that a particle of 1000 μm (1 mm) diameter requires about 320 years to reach a diameter of 100 nm. In the oceans, degradation can be assumed to proceed much slower due to limited availability of light, oxygen and bacteria

much slower due to limited availability of light, oxygen and bacteria. Microbial or photodegradation at the water surface thus may contribute to the formation of smaller particles, yet reaching the nanoscale may take a long time. We are not aware of studies showing NP formation due to these processes. Nano-fragmentation thus may involve two mechanisms; (1) direct nano-fragmentation may take place at the surface of macro- and microplastics (major process) and further gradual size-reductions may take place due to degradation (minor process). The different time scales of the two processes imply that embrittlement followed by physical abrasion of microplastics probably is the most important process explaining the formation of NPs.

12.2.2 Detection and Occurrence of Nanoplastic

We are not aware of studies reporting established analytical methods to detect nanoplastics in marine or freshwater. Under controlled conditions in the laboratory, several methods that apply to nanomaterials in general are also useful for nanoplastic fate and effect research, such as UV-VIS spectrometry, electron microscopy, field flow fractionation (FFF) or dynamic light scattering (DLS) techniques, each having their advantages and flaws (Von der Kammer et al. 2012). Shim et al. (2014) used SEM-EDS to confirm the presence of nanoplastics in abrasion experiments. In their effect study with mussels (*Mytilus edulis*), Wegner et al. (2012) used multiple wavelength UV-VIS as a proxy to detect pink-dyed nanoparticles and used dynamic light scattering (DLS) to track the actual size of the bioavailable aggregates over time. Velzeboer et al. (2014a) used transmission EM and conventional light microscopy to characterise pristine nanopolystyrene particles and aggregates, respectively. A recent study applied FFF coupled to multi-angle light scattering with pyrolysis to discriminate between various plastic types in spiked natural surface water samples (Kools et al. 2014).

Since separation, concentration and identification of NPs in environmental samples is still difficult, the actual occurrence of NPs is still a matter of speculation even though recent literature takes it as a fact. In his review, Andrady (2011) stated that there is little doubt that nanoscale particles are produced during weathering of plastic debris, but acknowledges that they are not yet quantified. The evidence is circumstantial in that abrasion indeed seems to show formation of nanoplastics in the laboratory (Shim et al. 2014). A study by Cózar et al. (2014) identified a deficiency of plastic particles at the lower end of an expected size distribution in the oceans and argued that nanofragmentation might be a plausible explanation for this deficiency.

12.3 Fate of Nanoplastic

Because NPs have not yet been measured in aquatic systems, only prognostic assessments of NP fate are possible. Freshwater carries plastics from land-based sources to the sea, which renders fate modelling of microplastics and NPs an

important area of research. In the literature, several processes have been identified as being important to address when modelling the fate of nanomaterials in freshwater, and a range of elaborate fate models are currently available (Gottschalk et al. 2013; Meesters et al. 2014; Quik et al. 2014). We argue that these models can be used for nanoplastics too, as long as some specific differences relating to densities, biofilm formation and attachment efficiencies are accounted for. For aquatic behaviour of nano-materials such as NPs, homo- and hetero-aggregation, advective flow, sedimentation, re-suspension, photo- and biodegradation, and sediment burial are important processes to consider (Quik et al. 2014; Besseling et al. 2014a). Velzeboer et al. (2014a) used pristine 60 nm polystyrene particles and observed a wide range of aggregate sizes, i.e. 199.3 ± 176.3 nm (range 100–500 nm) after 28 days, using TEM. Bhattacharya et al. (2010) measured substantial binding or heteroaggregation of 20 nm polystyrene particles with freshwater phytoplankton cells. Because of their low density, it is often assumed that substantial fractions of the total load of plastic particles from riverine sources reach the sea (Cózar et al. 2014; Wagner et al. 2014). However, it is plausible that organic matter fouling and subsequent hetero-aggregation with suspended solids, algae or detritus will cause settling and several recent reports indeed show presence of microplastics in the sediments (Zbyszewski and Corcoran 2011; Zbyszewski et al. 2014; Imhof et al. 2013; Wagner et al. 2014; Free et al. 2014). This process is relevant especially for NPs, because hetero-aggregation is particularly important at the nanometre-scale. In freshwater, burial is important to consider as it may be a loss process for nanomaterials from the biologically relevant sediment top layer (Koelmans et al. 2009). The loss processes including photo- or biodegradation have been discussed in the previous section. Besseling et al. (2014a) presented the first spatially explicit NP fate model that accounted for all the aforementioned processes. The model was implemented for a 40-km river stretch and showed the dependence of NP retention on nano- and microplastic particle size, density and attachment efficiencies. Simulations showed that settling of 100 nm NPs was stimulated by fast orthokinetic heteroaggregation, whereas for microplastics >0.1 mm Stokes settling dominated.

In marine systems, the same processes occur, although flow patterns, residence times and the nature of natural colloids and suspended solids (marine snow) are very different. Attachment efficiencies will be higher than in freshwaters due to the higher ionic strength. Collision frequencies however, will be lower due to much lower concentrations of natural colloids and solids in the water column. This trade-off has not yet been quantified for NPs. Wegner et al. (2012) were the first to measure and model the homoaggregation of 30 nm polystyrene particles in seawater and found rapid formation of 1000 nm aggregates within 16 minutes. Attachment efficiencies of 1 were required to explain the experimental observations (Wegner et al. 2012). Velzeboer et al. (2014a) used pristine 60 nm carboxylated polystyrene particles and observed a wide range of aggregate sizes, i.e. 361.1 ± 465.1 nm (TEM, range 100–500 nm) after 28 days (Fig. 12.3), which thus were larger than those observed in freshwater, as mentioned above. The 40 nm carboxylated polystyrene particles used by Della Torre et al. (2014) formed aggregates of 1764 ± 409 nm in natural seawater, whereas their 50 nm
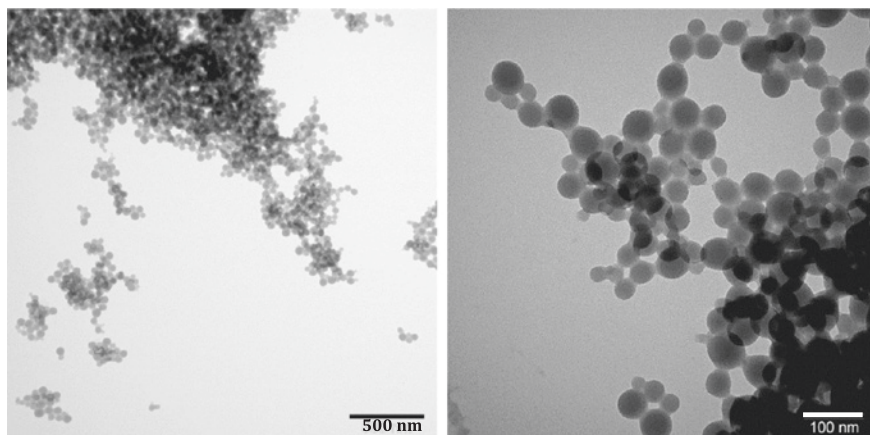
Fig. 12.3 Transmission electron microscopy images of 70 nm nano-sized polystyrene aggregates in freshwater (*left*) and seawater (*right*). Note that the TEM-based data may reflect exact in situ conditions to a lower extent because of the TEM preparation procedure. Reprinted with permission from Velzeboer et al. (2014a). © 2014 American Chemical Society

amino modified polystyrene remained dispersed at the nanoscale (89 ± 2 nm), although the authors report that these particles also partly aggregated with time. Another difference compared to freshwaters relates to the density of seawater, which is higher at lower temperature and higher salinity and thus increases with depth, an increase that additionally depends on season and location. The density of NP aggregates will also vary depending on polymer type, NP surface chemistry, extent of organic matter fouling and the thickness and nature of the biofilm once aggregates are formed. This means that settling of NP aggregates occurs until they reach seawater density and thereafter remain adrift in the water column (Cózar et al. 2014). Small changes in either aggregate or seawater density may cause slow upward or downward transport. Models that specifically simulate NP behaviour in the marine environment have not been published yet. However, because marine NP behaviour probably is behaviour of NP aggregates (Velzeboer et al. 2014a, b), Smoluchowski-Stokes based marine biogeochemical models can be applied such as those applied previously for settling of organic and mineral particles (e.g. Burd and Jackson 2009; Barkmann et al. 2010).

12.4 Bioaccumulation and Effects

12.4.1 Bioaccumulation and Effects of Nanoplastics

A handful of studies have investigated the accumulation or effects of NPs. As for membrane passage, Rossi et al. (2014) used molecular simulations to

assess the effect of nano-sized polystyrene on the properties of model biological membranes and concluded that the NPs could permeate easily into lipid membranes, which may affect cellular functions. Experimental validation would still be required to assess the actual relevance of this pathway. In this respect, Salvati et al. (2011) showed that carboxylated nanopolystyrene with sizes ranging from 40 to 50 nm entered cells irreversibly, by different endocytosis pathways. Inflammation responses have been observed in rat lung tissue in response to 64 nm polystyrene particles, showing that a low-toxicity material, such as polystyrene, can have inflammatory potential when present in nano-size (Brown et al. 2001). This study used an air-inhalation exposure scenario and the question remains to what extent this can be translated to aquatic systems, where aggregation would limit the concentrations of free NPs and direct inhalation of air-dispersed NPs does not occur. Bhattacharya et al. (2010) showed that adsorption of 1.8–6.5 mg/L of 20 nm polystyrene particles (yet present as agglomerates) hindered algal photosynthesis, possibly through reduction of light intensity and of air flow by the nanoparticles, and stimulated Reactive Oxygen Species (ROS) production. Ward and Kach (2009) showed that mussels (Mytilus edulis) and oysters (Crassostrea virginica) take up 100 nm PS beads, especially when incorporated into aggregates. They concluded that the direct bioavailability of freely dispersed NPs was very low and that capture and ingestion were the dominant exposure pathways for these species. Wegner et al. (2012) showed that mussels reduced their filter-feeding activity in response to 100 mg/L 30 nm nanopolystyrene. In two-generation chronic toxicity tests, Lee et al. (2013) showed nanopolystyrene ingestion by copepods (Tigriopus japonicus) and detected mortality of nauplii and copepodites for 50 nm (yet partly aggregated) polystyrene particles at concentrations of 12.5 mg/L (F0 generation) and 1.25 mg/L (next generation). Della Torre et al. (2014) observed severe developmental effects of amino-modified polystyrene nanoparticles in the early development of sea urchin (Paracentrotus lividus) embryos, with EC_{50} values of 3.85 and 2.61 mg/L at 24 and 48 h post fertilization. Kashiwada (2006) reported sorption of 39.4 nm nanopolystyrene to the chorion of medaka (Oryzias latipes) eggs and uptake into the yolk and gallbladder during embryonic development, whereas adults accumulated the NPs mainly in the gills and intestine yet also in the brain, testis, liver and blood. It was thus suggested that the NPs were capable of passing the blood–brain barrier. The acute (24 h) toxicity to medaka eggs was zero and 35.6 % for 1 and 30 mg/L NPs, respectively, although toxicity increased with higher salinity.

We are aware of three studies that use freshwater species. Cedervall et al. (2012) showed that 25 nm nanopolystyrene particles were transported through an aquatic food chain from green algae (Scenedesmus sp.), through water fleas (Daphnia magna) to carp (Carassius carassius) and other fishes, and affected lipid metabolism and behaviour of the fish. The effects were mechanistically explained from the chemistry and dynamics of the protein corona surrounding the NPs. Because it was a feeding study, effects could not be linked to NP concentration in the water. Casado et al. (2013) investigated the effects of 55 and 110 nm polyethyleneimine polystyrene nanoparticles on algae (Pseudokirchneriella subcapitata), crustaceans (Thamnocephalus platyurus; Daphnia magna), bacteria (Vibrio

fischeri) and rainbow trout (Oncorhynchus mykiss) cell lines (cytotoxicity). Effects were detected for the in vivo species with EC_{50} values between 0.54 and 5.2 mg/L, whereas EC_{50} values for cytotoxicity were between ~60 and 87 mg/L. Besseling et al. (2014b) reported that 70 nm polystyrene particles reduced the growth of algae (Scenedesmus obliquus) at high particle concentrations, and *malformed offspring* of *Daphnia* at a concentration of 32 mg/L. The effects on Daphnia were studied with and without fish (Perca fluviatilis) kairomones in the water and the effect of the kairomones appeared to be stronger in the presence of 1.8 mg/L nanoplastic. This suggests that nanoplastics might interfere with the *chemical communication* among species, which would cause subtle behavioural disturbances in finding a mate or food, or in the avoidance of predators such as fish. Such effects may be taking place at low concentrations that are not easy to detect using standard toxicological tests but that may result in changes in the food web in exposed ecosystems over time.

In summary, the limited literature provides some evidence of effects of NPs to marine and freshwater organisms, yet at relatively high concentrations, i.e. higher than ~0.5 mg/L NPs. There are currently no NP environmental concentrations to which this value can be compared, but the lowest NP effect concentration of 0.54 mg/L (Casado et al. 2013) is about four to six orders of magnitude higher than the 0.4–34 ng/L microplastic concentrations found in freshwaters in the USA (Eriksen et al. 2013) and Europe (Besseling et al. 2014c), but almost similar to the highest concentration estimated for marine water (i.e. 0.51 mg/L, see Besseling et al. 2014b; Lopez Lozano and Mouat 2009). However, because of the limited data, the uncertainties in these numbers and the absence of actual NP exposure data, these comparisons should be interpreted with caution.

12.4.2 Implications of Chemicals and Nanofillers Associated with Nanoplastics

Various kinds of additives are added during the manufacturing of plastics to increase its durability. Furthermore, residual monomers may remain in the plastic. For NPs in particular, the high surface area may cause exceptionally strong sorption affinities for 'external' toxic compounds (Velzeboer et al. 2014a), which implies that they will always be loaded with hydrophobic toxicants or trace metals (Rochman 2013a, 2014; Holmes et al. 2014). It can be hypothesized that the presence of such additives and absorbed chemicals might lead to increased exposure to these toxicants. In the laboratory, transfer and negative effects of such co-contaminants have indeed been shown upon ingestion of microplastic particles, but only in scenarios where clean organisms were exposed to plastics with rather high concentrations (Rochman et al. 2013b; Browne et al. 2013; Chua et al. 2014), thus forcing a maximum *fugacity gradient* upon the organism. Under more realistic natural exposure scenarios where organisms as well as the media water, sediment and plastic were brought at or close to equal chemical fugacity, no or

limited (i.e. within a factor of two) increases or decreases in chemical transfer of toxicants were found (Besseling et al. 2013). Several studies even showed beneficial effects of microplastic ingestion by *reducing bioaccumulation* due to sorption of chemicals to the plastic (Teuten et al. 2007; Gouin et al. 2011; Koelmans et al. 2013a, b; Chua et al. 2014). These different outcomes illustrate how the 'carrier effects' of microplastic depend on the initial boundary conditions of the test, which determine the direction of mass transfer between ingested or bio-accumulated plastic and tissue. This is consistent with recent model analyses that systematically explored these exposure scenarios (Gouin et al. 2011; Koelmans et al. 2013a, b, 2014b; Koelmans 2015). While the actual risk caused by chemical transfer due to microplastic ingestion may thus be of limited importance, exposure to NPs may still constitute a real hazard. Because of the surface effect, it may be possible that NPs retain organic toxic chemicals or heavy metals at higher concentrations than microplastics, thus leading to a *fugacity gradient to organism tissue* once ingested. If NPs are capable of permeating membranes, passing cell walls, translocate and/or reside in epithelial tissues for prolonged times (Kashiwada 2006; Cedervall et al. 2012; Rossi et al. 2014), the combination of particle and chemical toxicity may yield *unforeseen risks*. These hypotheses need to be experimentally validated, while also accounting for the possibly *low bioavailability* of NPs due to aggregation. During nanofragmentation, release of non-polymer nanoscale additives from the polymer nanocomposite product fragments may further add to the overall hazard (Nowack et al. 2012; Schlagenhauf et al. 2014). The smaller the additives, the better the improvement of polymer durability, which explains the addition of engineered nanoparticles such as carbon nanotubes (Grigoriadou et al. 2011; Bussière et al. 2013; Schlagenhauf et al. 2014). Although beneficial for their application, these additives increase the persistence of plastics in the environment and once degraded, may increase the overall risk due to an additional emission of nanomaterials.

12.5   Specific Challenges in Nanoplastic Effect Research

Several specific problems may arise when using NPs in aquatic tests or whole sediment toxicity tests with or without co-contaminants present. At present, it is not possible to detect NPs in the environment or to isolate sufficient quantities from the environment for effects research. This implies that manufactured NPs need to be used. This promotes uniformity of tests, but only commercially available polymer types (i.e. polystyrene beads) with limited size and shape (i.e. sphere only) can be tested, whereas NPs in the environment will include many different polymers of varying size and shape. Manufactured NPs may behave differently from environmental NPs because of these different properties. Manufactured NPs come with additives, monomers or oligomers of the component molecules of the plastics, or come with dispersants that are either deliberately added or that are just by-products of the manufacture process. Polystyrene, for instance, release styrene

monomers (Saido et al. 2014), which may add to the overall toxicity. If desired, such hydrophobic chemicals may be extracted from NP dispersions prior to testing, for instance using sequential Empore disk extractions (Koelmans et al. 2010). Commercial NPs are often delivered with a biocide to prevent bacterial growth during delivery and storage, which makes them useless for NP toxicity testing. Dispersants such as the surfactant sodium dodecyl sulphate (SDS) are often used. Although this helps to keep the NPs freely dispersed, dispersant concentrations should be kept far below toxicity thresholds and they should be included in the controls (Handy et al. 2012). Alternatively, the NPs can be dialysed towards clean water in order to reduce the concentrations of unwanted chemicals (e.g. Cedervall et al. 2012). NP surfaces are sometimes modified (functionalized) to maximize dispersion of otherwise hydrophobic NPs. This further raises the question what relevant exposure conditions are. On the one hand, a free dispersion may be preferred to achieve the level of control and constant nominal exposure concentration required from a regulatory perspective, and to obtain comparability of test results. On the other hand, a realistic test might aim at mimicking natural conditions as closely as possible, allowing for the formation of aggregates. All effect studies discussed in the previous section report the initial use of freely dispersed pristine NPs, yet acknowledge aggregate formation later on. This implies that aggregate formation and aggregate properties should be monitored during the tests. Several other challenges relating to the nanoscale of the particles are similar to those that were previously discussed for non-polymer manufactured nanomaterials (see Handy et al. 2012).

12.6 Implications and Recommendations

To date, the occurrence of NPs in the aquatic environment has not been proven and thus has to be considered a plausible hypothesis. Using manufactured NPs, some first effect tests have shown ingestion as well as negative effects of NPs on freshwater as well as marine species. Still, the toxicity thresholds seem higher than concentrations that are expected in the environment based on a worst-case assumption of conservative breakdown of microplastics present at currently known concentrations. However, we argue that potential impacts of NPs should not be considered in isolation. NPs might constitute an ecological stressor that adds to many other anthropogenic stressors such as trace metals, organic contaminants and non-polymer nanomaterials. Consequently, the question arises what contribution NPs make to the existing pool of other nano-sized materials. Natural nanoparticles have been shown to be ubiquitous in the environment, including hazardous ones (Wiesner et al. 2011). It has been suggested that engineered nanoparticles may account for only a negligible contribution to the concentrations of natural nanoparticles including soots, clays or other colloids that are already present (Koelmans et al. 2009). Future research may primarily focus on the sources, formation rates and exposure levels of NPs and on the fate of the particles in aquatic systems.

Methods to detect NPs in drinking and in natural waters are urgently needed. Prognostic screening-level effects tests may be performed in order to quantify the hazard once environmental concentrations are known. This research would benefit enormously from harmonisation and uniformity in classification of NPs and in methodologies used.

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