Invited Commentary

Ecotoxicity Testing of Microplastics: Considering the Heterogeneity of Physicochemical Properties

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EDITOR'S NOTE:

This is 1 of 15 invited commentaries in the series "Current Understanding of Risks Posed by Microplastics in the Environment." Each peer-reviewed commentary reflects the views and knowledge of international experts in this field and, collectively, inform our current understanding of microplastics fate and effects in the aquatic environment.

ABSTRACT

"Microplastic" is an umbrella term that covers many particle shapes, sizes, and polymer types, and as such the physical and chemical properties of environmental microplastics will differ from the primary microbeads commonly used for ecotoxicity testing. In the present article, we discuss the physical and chemical properties of microplastics that are potentially relevant to their ecotoxicity, including particle size, particle shape, crystallinity, surface chemistry, and polymer and additive composition. Overall, there is a need for a structured approach to the testing of different properties to identify which are the most relevant drivers of microplastic toxicity. In addition, the properties discussed will be influenced by and change depending on environmental conditions and degradation pathways. Future challenges include new technologies that will enter the plastic production cycle and the impact of these changes on the composition of environmental microplastics. *Integr Environ Assess Manag* 2017;13:470–475. © 2017 SETAC

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INTRODUCTION

Over the past few years, a number of laboratory studies have indicated that exposure to microplastics (MPs) can lead to adverse effects in test organisms. The majority of studies have mainly utilized primary microbeads of polyethylene (PE) and polystyrene (PS) to investigate particle ingestion and the resulting biological effects (see the recent review by Phuong et al. 2016). In addition, some studies have investigated the potential for trophic-level transfer (Farrell and Nelson 2013; Setälä et al. 2014). However, the term "microplastic" is used to describe a wide range of materials such as primary microbeads, secondary MPs, fibers, foams, granules, and fragments. In addition, the term "nanoplastic" is coming into common usage and can be included under the umbrella MP terminology. Thus, the physical and chemical properties of environmental MPs will differ from those of primary microbeads that are often used for laboratory ecotoxicity testing. Therefore, it is necessary to differentiate MPs in order to identify the potential hazards and risks they pose. Indeed, based on the experiences gained in the biological testing of engineered nanoparticles (ENPs), it has

been suggested that the observed effects of anthropogenic particles will depend on their physical and chemical properties, and not necessarily on the more traditional chemical parameter related to "dose by mass" (Oberdörster et al. 2005; Foss Hansen et al. 2007; Senjen and Hansen 2011). In the present article, we draw from the now-growing literature on the hazard assessment of ENPs to consider the information one needs to assist in describing environmental MPs from a physical (i.e., particle size, shape, surface area, and crystallinity) and chemical (i.e., chemical composition, including polymer type and additives) perspective. It is beyond the scope of the present article to make an in-depth review of exposure experiments; for this the reader should see, for instance, Phuong et al. (2016).

Understanding the multiple characteristics of microplastics

The physical and chemical properties that we will discuss here focus on particle size, particle shape, surface area, and crystallinity, as well as on chemical composition, including polymer type, additives compounds, and changes to surface properties (Figure 1).

Physical properties
Particle size. Particle size is an important property when considering how particles interact with biota (Montes-Burgos

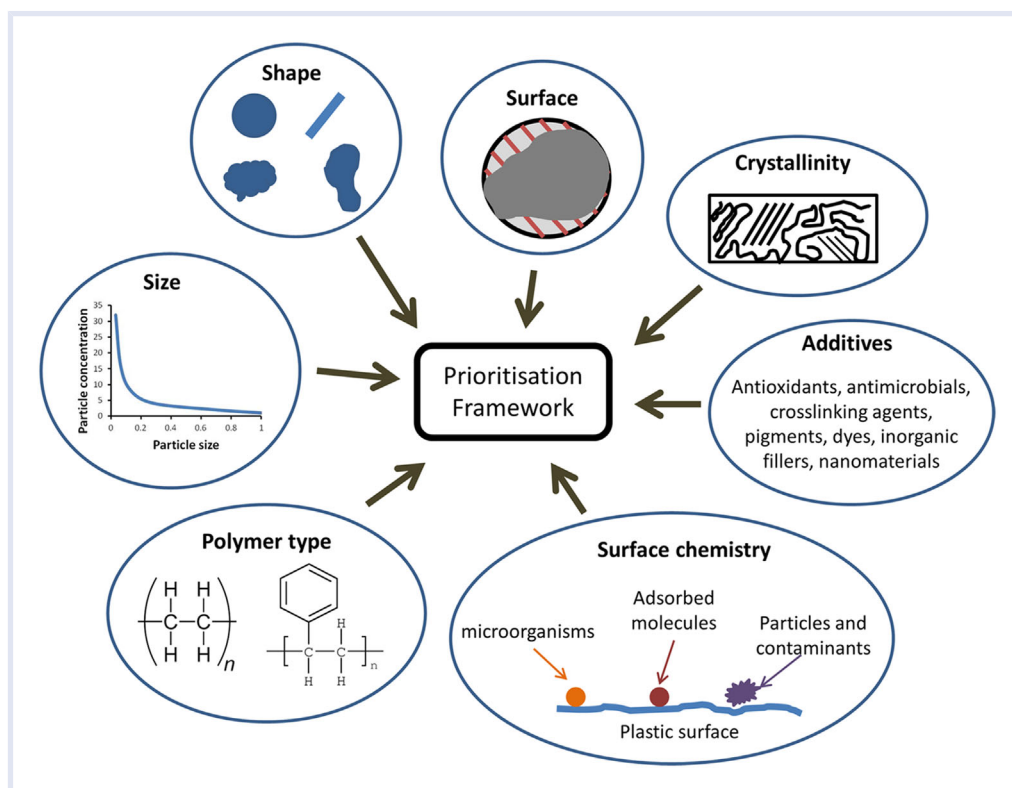* Address correspondence to lambert@em.uni-frankfurt.de

Figure 1. Different microplastic physical and chemical properties to be considered in a prioritization framework.

et al. 2010). Laboratory studies that have shown adverse responses of individual organisms to MP exposure generally use submicron and nanosized microbeads of uniform size and shape (e.g., Cole et al. 2013; Lee et al. 2013; Besseling et al. 2014). However, as environmental plastics weather and undergo processes of degradation, they will fragment and disintegrate, forming particles consisting of a broad particle-size distribution with a diverse range of particle shapes. The particle-size distributions of weathered MPs will be of a polydispersed nature that will increase in concentration with decreasing size distribution (Lambert and Wagner 2016). The maximum particle diameter that an organism can ingest will be determined by the morphology of the feeding and digestion apparatus of a particular species (e.g., Burns 1968; Rosenkranz et al. 2009).

Particle shape. Particle shape is another important property in determining the interaction of polymeric particles with biological systems (Wright et al. 2013). To highlight this, a recent study explored the effects of particle shape on the amphipod *Hyalella azteca*; the researchers observed a higher toxicity of polypropylene (PP) fibers compared to PP beads (Table 1; Au et al. 2015). A similar example can be found in the ENP literature. Zinc oxide nanosticks induced higher toxicity than did nanospheres in zebrafish embryos when endpoints of mortality and hatching inhibition were assessed (Hua et al. 2014). In both examples, particles with a more irregular or needle-like shape may attach more readily to internal and external surfaces and exert a greater effect.

Surface area. In terms of ENPs, surface area is considered an interesting parameter because it increases with decreasing particle size; therefore, nanoscale particles are expected to induce greater effects because of their greater overall surface area (Van Hoecke et al. 2008). Although surface area is not generally reported in MP studies, for primary microbeads it can be calculated based on spherical equivalent diameter, but for irregularly shaped secondary MPs this can cause an overestimation. For example, La Rocca et al. (2015) found that for nanoscale soot particles, surface area estimates using geometrical estimates can lead to a 7-fold overestimation of the surface area, and a particle shape factor needs to be applied to correct for this.

Polymer crystallinity. Crystallinity is an important polymer property because the crystalline region consists of more ordered and tightly structured polymer chains. This characteristic affects physical properties such as density and permeability, which in turn drives their hydration and swelling behavior. The crystallinity of environmental MPs will also change with degradation time. Here, the preferential degradation in the amorphous region of the polymer will cause the overall crystallinity to increase (Gopferich 1996; Chen et al. 2000) as the MP decreases in size. This process will result in the formation of crystallites, which might differ in toxicity compared to the parent MP. Changes in crystallinity will make environmental MPs very different from their microbead counterparts, and will influence other physical (e.g., surface area, particle shape, particle size, and density) and chemical (e.g., leaching of additives, adsorption of

Table 1. Selected laboratory studies cited in the present article, particle types, and observed responses

Organism	Particle type	Main findings	Reference
Hyallela azteca	PE MPs (powder; size: 10 and 27 μm) PP fibers (secondary; length 20–75 μm; dia. 20 μm)	Fibers were found to be more toxic than particles with 10-d LC50 of 71.43 fibers per mL compared to 4.64×10^4 particles per mL.	Au et al. 2015
Copepod (*Centropages typicus*) collected from western English coast, Plymouth, UK	PS microspheres Size: 0.4–30.6 μm	The presence of 7.3 μm at >4000 mL/L beads reduced algae feeding; larger PS beads showed no impact on feeding.	Cole et al. 2013
Tigriopus japonicus	PS microspheres Size: 0.05, 0.5, and 6 μm	6-μm beads did not affect the survival over 2 generations; 0.05- and 0.5-μm beads caused increased toxicity and impacts on survival and development in the F_1 generation at 9.1×10^{11} and 9.1×10^8 per mL.	Lee et al. 2013
Daphnia magna	PS carboxylated microspheres Size: 0.02 and 1 μm	Demonstrated ingestion of both bead sizes, but the 20-nm beads were retained to a greater degree within the organism.	Rosenkranz et al. 2009
Daphnia magna	PS microspheres Size: 70 nm	Reduction in body size and lower reproduction at concentrations $\geq$30 mg/L (using a nominal density of 1.05 g/cm^3, this equals 74.3 billion particles per L).	Besseling et al. 2014
Eurytemora affinis *Neomysis integer*	PS microspheres Size: 10 μm	Potential of transfer from meso- to macro-zooplankton at concentrations of 1000, 2000, and 10 000 particles per mL.	Setälä et al. 2014
Mytilus edulis *Carcinus maenas*	PS microspheres Size: 0.5 μm	The number of MPs in the hemolymph of the crabs was highest at 24 h (15 033 per mL), and was almost gone after 21 d (267 per mL).	Farrell and Nelson 2013
Zebrafish embryos (*Danio rerio*)	Uncoated Zn oxide nanospheres (43 nm), nanosticks (150 nm), and cuboidal particles (900 nm)	Zn oxide nanosticks induced higher toxicity than nanospheres and cuboidal particles for mortality and hatching inhibition endpoints.	Hua et al. 2014
Pseudokirchneriella subcapitata	Silica nanospheres Size: 12.5 and 27 nm	Particle size and surface area determine ecotoxicity potential and not mass.	Van Hoecke et al. 2008

MP = microplastic; PE = polyethylene; PP = polypropylene; PS = polystyrene; UK = United Kingdom.

pollutants) properties, in turn affecting ingestion rates and effect outcomes.

Chemical properties

Polymer type and additives. Plastic-associated toxicity may be caused by the leaching of chemicals, including residual monomers, starting substances, solvents, and catalysts, as well as additives (e.g., antioxidants, dyes, biocides, plasticizers) incorporated during compounding and processing (Muncke 2009; Andrady 2015). The toxicological profiles of several monomers and additives used in the production of certain plastic types are well known. With regard to polymer type, examples include the following:

1) Plasticized polyvinyl chloride (PVC), often considered the most hazardous plastic because of its high chloride and additive content, and the formation of dioxins during manufacturing and incineration processes (Rossi and Lent 2006)

2) Polycarbonate, manufactured from bisphenol A, which is an endocrine-disrupting compound

3) Polyacrylonitriles and acrylonitrile butadiene styrene, based on classifications within the European Union classification, labeling, and packaging (CLP) regulation (Lithner et al. 2011).

4) Polystyrene and its copolymers because, again, the monomer styrene is a suspected carcinogen (Rossi and Lent 2006; Lithner et al. 2011). In addition, PS is of interest because its styrene oligomers are shown to leach into ambient water and sediments (Kwon et al. 2015).

5) Polyurethanes and epoxy resins, based on their monomer classifications within the European Union CLP regulation (Lithner et al. 2011).

6) Polyethylene terephthalate (PET) because it is suspected to leach endocrine-disrupting chemicals (Wagner and Oehlmann 2009, 2011).

The environmental release of additives from plastic materials and other plastic-associated compounds can potentially occur

at all lifecycle stages, depending upon how a particular chemical is compounded within a particular polymer matrix (Lambert et al. 2014). For example, low molecular weight additives that are weakly embedded in the polymer matrix that readily migrate, such as flame retardants from television housings and other electronic items (Kim et al. 2006; Deng et al. 2007), Pb from unplasticized PVC pipes (Al-Malack 2001), nonylphenol from food contact materials (Fernandes et al. 2008), extractable PET cyclic and linear oligomers from bottles and food trays (Kim and Lee 2012), and leaching of Sb from PET water bottles (Shotyk and Krachler 2007; Westerhoff et al. 2008; Keresztes et al. 2009).

Overall, the rate at which residual monomers and additives leach will depend on physical properties such as the pore diameter of a particular polymer structure and the molecular size of the monomer and additives used (Gopferich 1996). The relevance of leachable chemicals for the hazard potential of MPs will depend on their concentration in the parent plastic, their partitioning coefficient, and the age and degree of degradation of a specific MP. For example, an aged MP may have a higher degree of cystallinity, which may result in a reduced leaching.

Surface chemistry. The surface chemistry of environmental MPs will also change as they age. Photo and oxidative degradation processes will affect the plastic surface by creating new functional groups through reactions with OH radicals, O, N oxides, and other photo-generated radicals (Chandra and Rustgi 1998). An increase in chemical reactions then causes the surface of a plastic to crack, opening up new surfaces for further degradation processes to occur (Lambert et al. 2013). These processes may weaken the plastic surface, causing the further release of microscopic particles upon ingestion, enhancing chemical leaching, and increasing gut retention times through the formation of more angular-shaped particles, making environmental MPs distinctly different from primary microbeads. In addition, changes to surface chemistry play an important role in influencing the interactions between particles and biota (Gerritsen and Porter 1982), and will make the surface of the plastic material more accessible for microorganisms because they are better able to utilize the oxygenated functional groups (Roy et al. 2008). Studies with *Daphnia magna* have also shown that surface chemistry influences the uptake, retention, and internalization of quantum dots (Feswick et al. 2013), and the surface chemistry is also thought to play an important role in cell uptake and translocation of fullerenes (Ke and Lamm 2011). So far, however, it is unknown whether these differences in surface chemistry are important determinants of toxicity in environmentally realistic exposure scenarios.

Future challenges

It will be important to recognize that new polymer types and additives will enter the plastic production cycle in the future due to technological innovations. There is already an increase in the application of polymers based on biological resources. For example, polylactic acid (PLA) is almost on the verge of entering

into bulk production, while polyhydroxyalkanoates (PHA) production is between the pilot plant and commercial stages (Amulya et al. 2015; Mohan 2016). In particular, PHA is often cited as a sustainable alternative and is considered fully biodegradable, but like its petrochemical counterparts, to be made fit-for-purpose it will need to be processed with additive compounds. Here, public education is needed to ensure that plastics made from biological C sources are not considered appropriate to litter, and toxicological testing of new materials will be needed as well.

The next important development for the plastics industry is the utilization of ENPs. Already, a 7% share of nano-composites among plastics in the USA is predicted by 2020 (Roes et al. 2012). At the nanoscale, the physical and chemical properties of a material differ from the ones of the bulk matter counterpart. This creates the potential to exploit these new properties, for example, plastics re-inforced with nanofillers (nanoclay) and nanosilica for weight reduction, C nanotubes for improved mechanical strength, and nano-Ag utilized as an antimicrobial agent in plastic food-packaging materials. How ENPs will affect the physical and chemical properties of MPs is a question that remains unanswered, but this may depend on how they are compounded within the material and the concentrations that are used. Some research suggests that ENPs incorporated into plastics are not necessarily released as single ENPs. Wohlleben et al. (2011) reported that no free ENPs were liberated during artificial weathering of polymeric nanocomposites. Liu et al. (2012) studied the degradation of an acrylate polymer with embedded quantum dots, and found the leachates contained no free ENPs. This is because in many applications ENPs are embedded in a matrix and any release will occur through the release of matrix-bound ENPs (Koehler et al. 2008). This suggests that fragmentation of the parent plastics into MPs will produce a more complex MP–nanocomposite particle with physical and chemical properties that are very different from MPs recovered in current monitoring studies.

CONCLUSIONS

To summarize, there are many challenges associated with investigating the environmental impacts of micro- and nanoplastics, and some of these challenges mirror those associated with ENPs. In the present article, we have highlighted some of the major physical and chemical factors relevant to the hazard characterization of MPs. However, it is still open to question which of the discussed properties drive the ecotoxicity of a particular type of MP. In addition, these properties will be influenced and will change, depending on environmental conditions and degradation pathways. As the ecotoxicological research on MPs evolves, different physicochemical properties should be taken into account rather than focusing exclusively on microbeads made from PS. This will help to identify the physical and chemical properties most relevant for the environmental impacts and, in turn to prioritize polymers for testing and assessment.

Data availability—Data are available by contacting corresponding author Scott Lambert (lambert@em.uni-frankfurt.de).

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