Critical Review

Microplastics in the Aquatic Environment: Evidence for or Against Adverse Impacts and Major Knowledge Gaps

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Abstract: There is increasing scientific and public concern over the presence of microplastics in the natural environment. We present the results of a systematic review of the literature to assess the weight of evidence for microplastics causing environmental harm. We conclude that microplastics do occur in surface water and sediments. Fragments and fibers predominate, with beads making up only a small proportion of the detected microplastic types. Concentrations detected are orders of magnitude lower than those reported to affect endpoints such as biochemistry, feeding, reproduction, growth, tissue inflammation and mortality in organisms. The evidence for microplastics acting as a vector for hydrophobic organic compounds to accumulate in organisms is also weak. The available data therefore suggest that these materials are not causing harm to the environment. There is, however, a mismatch between the particle types, size ranges, and concentrations of microplastics used in laboratory tests and those measured in the environment. Select environmental compartments have also received limited attention. There is an urgent need for studies that address this mismatch by performing high quality and more holistic monitoring studies alongside more environmentally realistic effects studies. Only then will we be able to fully characterize risks of microplastics to the environment to support the introduction of regulatory controls that can make a real positive difference to environmental quality. *Environ Toxicol Chem* 2018;37:2776–2796. © 2018 SETAC

Keywords: Microplastics; Species sensitivity distribution; Risk; Persistent organic pollutants

INTRODUCTION

Over the past decade there has been increasing scientific, public, and regulatory interest in the occurrence and impacts of microplastic in the environment, which have been defined as plastic particles <5 mm in size (Hidalgo-Ruz et al. 2013) arising from a number of sources including cosmetics, abrasion of larger items through use (such as tire fragments), and the fragmentation of larger items of plastic (Sundt et al. 2014). In 2010, fewer than 10 peer-reviewed articles contained the word "microplastic," although this number had risen to approximately 306 in 2017. Alongside this, there have been significant policy and regulatory developments around the use and emissions of microplastics, for example, in the United States, the Microbead Free Water Act of 2015 and the Environmental Protection (Microbeads; England) Regulations 2017, which announced a ban on the use of microbeads in all wash-off cosmetic products.

These regulatory interests are being driven by the increasing evidence that microplastics occur in the environment (Lusher 2015), are taken up into organisms (Eerkes-Medrano et al. 2015), and the perception among many that the materials are adversely affecting marine life and that they may pose a risk to human health (Cole et al. 2011; Wright et al. 2013; Ivar do Sul et al. 2014; Van Cauwenberghe et al. 2015; Eerkes-Medrano et al. 2015; Galloway 2015; Koelmans et al. 2015; Lassen et al. 2015; Lusher 2015; Oberbeckmann et al. 2015; Duis and Coors 2016; Auta et al. 2017; Anbumani and Kakkar 2018). However, various researchers have raised concerns over the quality of some of the studies (Song et al. 2014; Phuong et al. 2016; Connors et al. 2017; Lusher et al. 2017), and little effort has been made to put the findings from different studies of the environmental occurrence and effects of microplastics into a risk context (Koelmans et al. 2017).

Therefore, we present the results from a systematic review of the published literature to attempt to answer the following question: do existing data on the occurrence and effects of microplastics in the environment indicate that these materials are causing harm? In answering this question, we explore the evidence base for a number of assertions made by the broader community concerning microplastics in the environment,

including "wastewater treatment processes are unable to remove microplastics," "microplastics occur in waters and sediment," "microplastics are taken up by organisms," "microplastics can act as vectors of persistent organic pollutants into organisms and through food chains," and "microplastics are adversely affecting organisms in the environment." We also identify major knowledge gaps that need to be addressed to establish the extent of microplastic environmental impacts. It is our hope that the results of the analysis will help to focus future research efforts on the impacts of microplastics in the environment.

METHODS

A systematic review was conducted of papers published up to the end of 2017, which were identified by the search engines Scopus and Web of Science. The search terms "microplastic" and "environment" were used and 320 peer-reviewed research articles identified. Further targeted searching was conducted when cited literature yielded relevant peer-reviewed articles and applicable reports published by government agencies that were missed by the search engine.

To allow comparison of data from different sources, ecotoxicity studies reporting concentrations in mass per liter were converted to particles per liter according to the method of Connors et al. (2017). Aquatic measured environmental concentrations (MECs; freshwater and marine) were converted from particles per cubic meter to particles per liter by dividing by a factor of 1000. The MECs that were reported in particles per square meter were not converted to particles per liter and subsequently not included in assessments. A species sensitivity distribution (SSD) was created using the US Environmental Protection Agency's CADDIS Species Sensitivity Distribution Generator (US Environmental Protection Agency 2014), also used in a recent study to build SSDs for engineered nanoparticles (Garner et al. 2015). Ecotoxicity and occurrence data for marine and freshwater species/environments were included. Ecotoxicity endpoints included were limited to mortality, growth, and reproduction (Connors et al. 2017), and both no-observed-effect concentrations (NOECs) and lowest-observed-effect concentrations (LOECs) were included. Ecotoxicity data included were limited to 10- to 5000-µm particle size exposures because this reflects the smallest size fraction identified in environmental samples with commonly used spectrometric methods (Löder and Gerdts 2015; Song et al. 2015) and the upper microplastic size limit. Ecotoxicity data used to build the SSD are listed in the Supplemental Data.

SOURCES AND OCCURRENCE OF MICROPLASTICS IN THE ENVIRONMENT

In the present review, we use the definition of "plastic" described by the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (2015), which defines a plastic as a synthetic water-insoluble polymer, generally of petrochemical origin, that can be molded on heating and manipulated into various shapes designed to be maintained during use (see also Lassen et al. 2015). This includes both thermoplastics, such as polyethylene and polypropylene, and thermosets (i.e., cannot be remolded after successive heating), for example, polyurethane foams and epoxy resins (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection 2015). A microplastic is any solid plastic particle ≤5 mm in size (Eerkes-Medrano et al. 2015). Agreement on the higher end of the microplastic range (5 mm) is consistent in the literature; however, various authors have proposed differing lower limits (Hidalgo-Ruz et al. 2013; Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection 2015; Lassen et al. 2015). This generally coincides with particle sampling size limitations (Barrows et al. 2017) or analytical limits of detection (Löder and Gerdts 2015; Shim et al. 2017). For example, the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (2015) set the lower limit of the microplastic size range to 1 nm, whereas Lassen et al. (2015) limited the lower end of the range to 1 µm. Standardization of the microplastic size range would be useful, as would agreement on subclassifications of particle size. For example, as particles become smaller, especially in the nanometer size range, they are expected to behave differently from their larger counterparts, which can influence environmental transport or fate (Besseling et al. 2017) and potentially increase the likelihood of adverse effects on exposed organisms (Jeong et al. 2016).

In the environment, microplastics are classified as either primary or secondary, depending on their source. Primary microplastics are used intentionally in the ≤5-mm size range and include cosmetic beads that are used in scrubs and shampoos, particles used for sandblasting and preproduction resin pellets (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection 2015; Duis and Coors 2016). Secondary microplastics are fragments of larger plastic materials degraded through either use (e.g., release of fibers from washing clothing or textiles), waste management, or fragmentation of larger plastic in the natural environment (e.g., plastic bags or bottles; Lassen et al. 2015).

Little is known about the emission rates of these microplastic sources to the environment, and a detailed analysis of the current knowledge in this area is beyond the scope of the present review. Briefly, the focus thus far has been on primary microplastics (Lebreton et al. 2017). This is likely because usage/sales volume multiplied by microplastic content enables a rough emission estimation for down-the-drain microplastics, which are expected to enter the environment through wastewater treatment plants (WWTPs; Sundt et al. 2014). Less is known about the formation rate of secondary microplastics because this is influenced collectively by several factors such as polymer type and environmental exposure conditions (Song et al. 2017). Fragmentation can be aided by biotic activity, for example, microbial degradation or animal activity (Sundt et al. 2014), although photodegradation will also fragment plastic particles at variable rates depending on the surrounding environment (e.g., temperature, water depth) and mechanical weathering is also possible (Cooper and Corcoran 2010). How these factors operate together is poorly understood, making exposure assessments of secondary microplastics difficult (Ter Halle

et al. 2017). In contrast, microplastics in cosmetic products have received more attention (Gouin et al. 2015). Sundt et al. (2014) attempted a detailed assessment of all primary and secondary microplastic emissions for Norway and concluded that tire dust was expected to be the largest contributor to microplastic concentrations in the Baltic Sea, whereas consumer products were expected to have the smallest contribution. A similar conclusion was drawn for emission estimations of microplastics in Denmark: 0.9% of the total microplastic emission to the aquatic environment was expected to be primary microplastics (0.1% cosmetic products), while tire dust was expected to contribute 60% of the total microplastic emission to the aquatic environment (Lassen et al. 2015). Eunomia (2016) also came to a similar conclusion, where land-based microplastic emissions to the marine environment were dominated by tire dust. Eunomia (2016) also reported the relative contribution of inland, coastal, and at-sea activities on total plastic entering the marine environment as 0.5, 9, and 1.75 million tonnes, respectively. As these emission estimates develop for both primary and secondary microplastics to marine, freshwater, and terrestrial systems, they can be paired with models (Besseling et al. 2017; Horton et al. 2017) that can estimate how particle size and source (e.g., wastewater effluent) impact microplastic environmental fate and occurrence.

Microplastic environmental occurrence

We identified 109 studies reporting MECs of microplastics in the environment. These studies focused on sampling freshwater, marine water, and sediment. Data for terrestrial soils are virtually nonexistent (Lwanga et al. 2017), despite agricultural microplastic sources or spreading of WWTP biosolids for agriculture, as well as land-based waste disposal being potential sources of microplastics in agricultural soils (Wagner et al. 2014). In the present section, we summarize the analytical methods used and the results obtained in terms of microplastic concentrations and characteristics.

Methods of microplastic sampling and analysis. The majority of monitoring studies (42%) employed solely visual identification methods (i.e., naked eye or dissecting microscopes), with 43% of those studies published in 2016 and 2017 (Figure 1). Visual identification only permits identification down to 500 µm (Löder and Gerdts 2015). Although visual confirmation techniques are inexpensive in terms of time and cost, misidentification of natural particles such as coal ash or coal fly (Eriksen et al. 2013), quartz or calcium carbonate (Ballent et al. 2016), or steric acid and castor oil (Ziajahromi et al. 2017b) is possible. Several authors have therefore concluded that the visual identification error rate for identifying natural particles as microplastics is unacceptably high, ranging from 33 to 70% (Hidalgo-Ruz et al. 2013; Dekiff et al. 2014; Lenz et al. 2015; Lusher et al. 2015; Ballent et al. 2016; Clunies-Ross et al. 2016; Fischer et al. 2016; Horton et al. 2017; Imhof et al. 2017; Kanhai et al. 2017). Studies not using appropriate analytical confirmation techniques are likely overestimating environmental concentrations of relevant size fractions (Lusher et al. 2017). This is
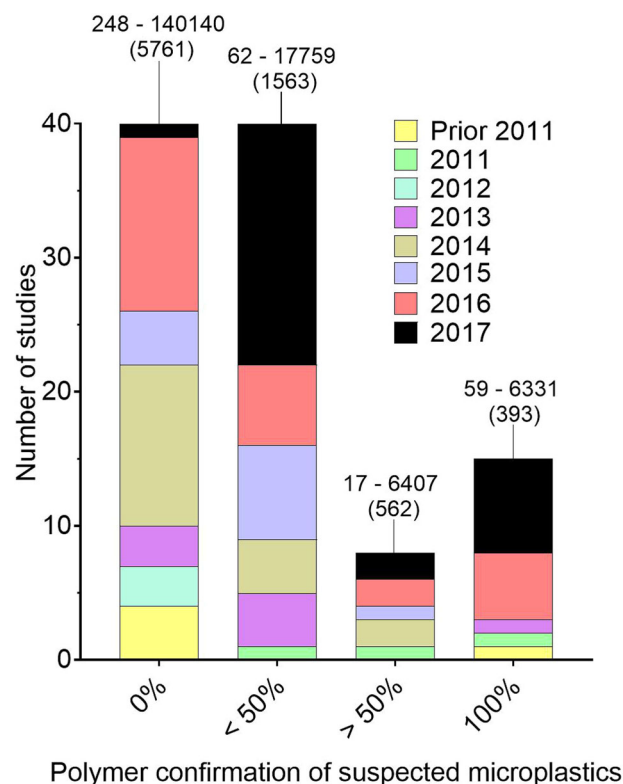

FIGURE 1: Percentage of suspected microplastics per study subject to polymer identification using analytical techniques such as Raman and Fourier transform infrared spectroscopy, with 0% indicating that only visual analysis techniques were used to identify microplastics. The total particle count for studies in each category is also provided as the range (i.e., the study identifying the fewest and greatest numbers of microplastic particles) and average. In addition, 63, 60, 38, and 53% of studies did not report a total number of particles found in the 0, <50, >50, and 100% polymer identification categories, respectively.

especially true for fibers, where visual analysis alone cannot differentiate between cotton or other natural fibrous materials and those of synthetic origin (Fischer et al. 2016). It is also evident from Figure 1 that the total microplastic particle count ranges substantially among studies, 17 to over 100 000 pieces, which is likely the result of sampling location, effort, and method.

Advanced analytical confirmation methods (some form of Raman scattering or [µ]-Fourier transform infrared spectroscopy [FTIR]), which allow particles to be characterized in terms of their chemical makeup and hence to distinguish from natural particles and identify polymer type, were used in 58% of the studies. The use of various Raman and FTIR spectroscopy techniques can also lower the particle size detection limit to 1 and 10 µm, respectively (Löder and Gerdts 2015; Song et al. 2015; Duis and Coors 2016); however, confidence in detection is decreased at <131 µm (Frère et al. 2017). In 64% of the studies involving confirmation methods, confirmation was performed on <50% of particles sampled. A further 13% used a chemical identification technique to identify >50% of particles sampled, whereas 23% confirmed 100% of suspected microplastics (Figure 1). Confirmation of >50% of suspect microplastics was not limited to studies with low total particle counts (e.g., <500) despite the additional cost and effort for sample analysis. Similar to the

studies using visual techniques, MECs from any study where <50% of suspect microplastics have been confirmed, should be treated with caution.

Problems can also be encountered in microplastic detection when using appropriate analytical confirmation methods because of difficulties pertaining to particle brittleness (breaking apart in the sample preparation stage), biofouling of particles (interfering with the signal), or the particle size being too small to be adequately analyzed (Leslie et al. 2017; Shim et al. 2017).

Occurrence in surface water. Surface water monitoring for microplastics has been performed on all continents (Figure 2). The majority of studies that have monitored microplastics in the water column have focused on oceans and seas ($n = 58$), with only a handful focusing on freshwater ($n = 10$; Figure 2). The studies report results in different units of items per square meter and items per cubic meter, which are incompatible, and a conversion between the 2 is not straightforward (Isobe et al. 2015). Reporting in items per square meter diminishes the usefulness of occurrence data because all ecotoxicity tests are reported in terms of mass or particles per volume (Duis and Coors 2016). Despite this, studies still report only items per square meter (Ruiz-Orejon et al. 2016; Sutton et al. 2016; Imhof et al. 2017; Nel et al. 2017), highlighting the fact there is still a need for standardization of reporting. The sampling methods employed will also affect results (Lusher et al. 2014). A study comparing several commonly used sampling methodologies found that concentrations differed by orders of magnitude
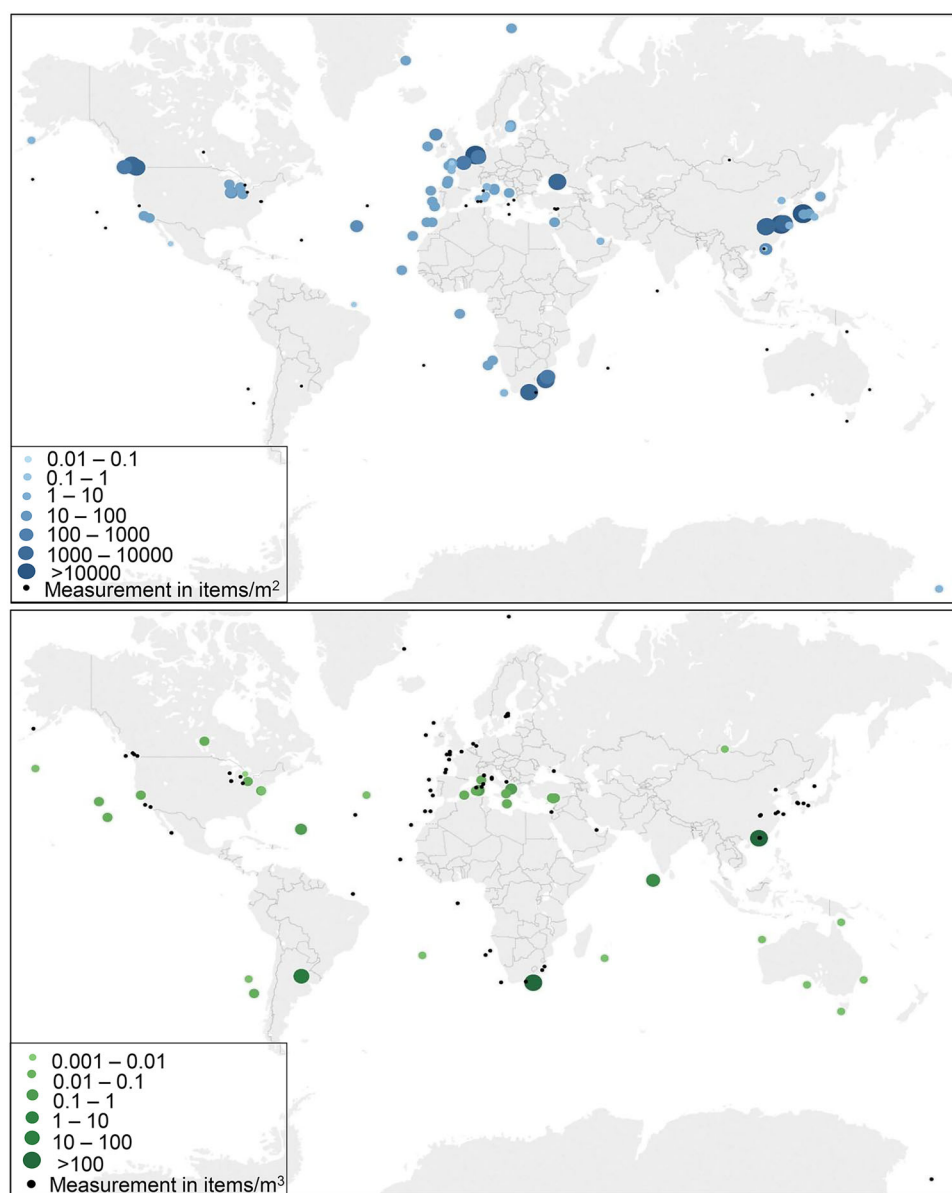

FIGURE 2: Global distribution of marine and freshwater aquatic measured environmental concentrations (MECs) from the reviewed literature (see Supplemental Data for references). Reported units were not converted, and therefore, relevant MECs are reported in 2 separate maps: items per cubic meter (top) and items per square meter (bottom). Black dots represent concentrations reported using the other unit (e.g., in the items/m^3 map (top) black dots are where concentrations in items/m^2 have been reported).

depending on the method used (Song et al. 2014). This is attributable to the depth at which sampling was focused or particle size sampling limitations imposed by net mesh sizes. In contrast, methods that collect whole water samples (such as grab sampling) will not discriminate based on particle size (Barrows et al. 2017). Again, standardization is needed to produce repeatable and comparable monitoring results (Hidalgo-Ruz et al. 2013).

There is reasonable global coverage from the 16 yr of occurrence data we have reviewed (Figure 2). Highest concentrations have been reported near heavily urbanized and industrialized coastal areas and in rivers, with the highest MECs being reported in the canals of Amsterdam (100 000 items/m^3 [100 items/L]; Leslie et al. 2017) and off the South Korean coast (16 727 items/m^3 [16.7 items/L]; Song et al. 2015). This observation is supported by a recent extensive modeling exercise which identified rivers passing through heavily industrialized areas in Asia as one of the largest freshwater contributors to oceanic microplastic loads (Lebreton et al. 2017).

Occurrence in sediment. Fifty monitoring studies quantified microplastics in marine/coastal sediments, with only 10 studies investigating occurrence in freshwater sediments (Figure 3). Similarly to the water column monitoring studies, these investigations report occurrence in different units (i.e., items per kilogram, items per square meter, and items per cubic meter; Figure 3). Although it can be possible to convert between units, the methodological details to achieve this are not always reported (Van Cauwenberghe et al. 2015). The majority of reported sediment monitoring studies were performed in Europe, and similar to the aqueous occurrence studies, a greater focus has been on beach and nearshore sediment (Figure 3). Freshwater sediment samples came mainly from lakes (66%). Highest concentrations were reported in the Taiwan Strait (42 560 items/m^3; Kunz et al. 2016).

Microplastic type and chemical characterization. Sample morphological composition was reported for sediment and the water column (marine and freshwater) in terms of sample concentration or percentage in 83% of the occurrence studies reviewed (Figure 4A and B). The overall average sample composition in the water column was 52% fibers, followed by 29% fragments, with other particle morphologies including beads/spherules, films, foams, and others making up only a small proportion of the overall microplastics detected. A similar trend was observed in sediment where fibers made up 45% of the particles followed by fragments which made up 33% (Figure 4B). In terms of polymer type, trends were also similar in the water column and sediment, with the greatest proportion of particles comprised of polyethylene, followed by polyethylene terephthalate and polyacrylamide in water (Figure 4C) and polypropylene in sediment (Figure 4B). Distributions of percentage compositions for different particle types seen in the sediment and water column monitoring data are summarized in Figure 4E and F.

Microparticles do occur in surface waters and sediments around the world. It is, however, difficult to define the precise degree of exposure in different regions and environmental matrices because of variability/challenges in sampling techniques (Song et al. 2014), differences in the microplastic detection methods used (Löder and Gerdts 2015), ways in which microplastics in samples have been categorized (Helm 2017), differences in sampling design (Underwood et al. 2017), reporting units, and surveyed particle sizes (Phuong et al. 2016; Barrows et al. 2017). Standardization is imperative in the future to allow comparison of results across monitoring studies and also with data from effects studies.

Where microplastic characterization has been done, the majority of microplastics detected in monitoring studies are believed to be of secondary origin (i.e., fragments of larger plastic items that have degraded or fibers unintentionally released from clothing), which indicates that sources of secondary microplastics will be important to understand if policy or mitigation measures to reduce microplastics in the environment are to be effective. A great deal of regulatory focus has been placed on primary microplastics, which, in terms of occurrence, appear to be less significant based on the present results. Therefore, reducing or banning (e.g., cosmetic microbeads) may only have a limited impact on reducing environmental microplastic loads, a conclusion also drawn by Gouin et al. (2015). Tracing the source of secondary microplastics is more complex than that of primary microplastics, which may be why they have evaded focus so far. Therefore, reporting sample composition is important to help identify which particles are of highest priority for ecotoxicity testing and evaluation of their sources and pathways. The majority of data plotted in Figure 4 pertains to the marine environment (water column and sediment); however, the environmental distribution of microplastics (polymer type and morphology) will vary based on microplastic and environmental characteristics. Therefore, as more data become available for other compartments, such as freshwater and the sea surface layer, these data should be presented separately to better characterize microplastic distribution and exposure in various environmental compartments.

Are WWTPs significant sources of microplastics?

It is believed that WWTPs are a significant contributor of microplastics to the environment, and it has been suggested that they remove little or none of the microplastics that are emitted to the wastewater system because of their small size (Browne et al. 2011; McCormick et al. 2014). Detecting microplastics and estimating WWTP removal presents many challenges; for example, biofouling is highly likely, and many cellulosic fibers (e.g., toilet paper) are present, resulting in the possibility of a high percentage of misidentifications (e.g., Ziajahromi et al. 2017b). Ideally, therefore, when performing monitoring of microplastics in WWTPs, all suspected microplastics should be subject to analytical confirmation (Tagg et al. 2015; Dyachenko et al. 2017). Furthermore, robust sampling approaches are needed to capture daily variations in flow and WWTP residence times because significant differences have been found in samples taken throughout the day (Leslie et al. 2017).
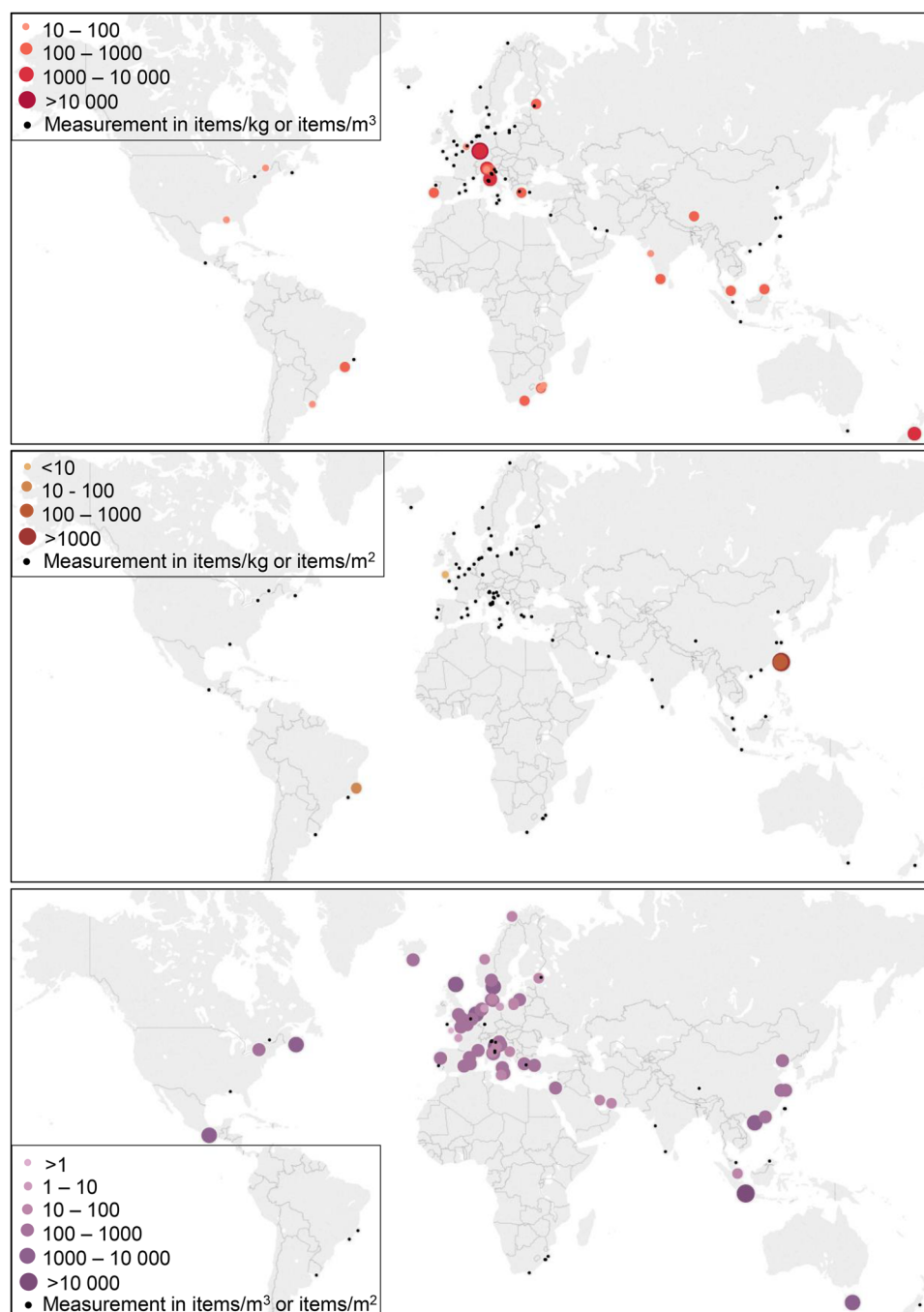
FIGURE 3: Global distribution of marine and freshwater sediment measured environmental concentrations (MECs) from the reviewed literature (see Supplemental Data for references). Reported units were not converted, and therefore, relevant MECs are reported in 3 separate maps, items per square meter (top), items per cubic meter (middle), and items per kilogram (bottom). Black dots represent concentrations reported using the other units (e.g., in the items/m^2 map (top) black dots are where concentrations in items/m^3 or items/kg have been reported).

A number of studies have been performed that have quantified the removal of microplastics in different wastewater treatment processes (Table 1). Primary treatment alone can remove an average of 65% of the total microplastic influent load, whereas secondary and tertiary treatment options can remove an average of 94% of the total influent load (Table 1). A study of Danish WWTPs predicted environmental emission rates of 0.3% of the incoming microplastic mass (Volertsen and Hansen 2017). The majority of microplastics that have been detected in WWTP effluent are plastic fibers and fragments, with only a small proportion comprising microbeads, even though microbeads are the focus of regulatory concern. The observed removal of microplastics is explained by the fact that even though they can move through the exclusion meshes, many are likely to float because of their density and be subsequently removed in the grease layer (Murphy et al. 2016) by skimmers (Carr et al. 2016) in the primary treatment process. If the microplastic is not floating, it is likely fouled and will either sink to the bottom of a settling
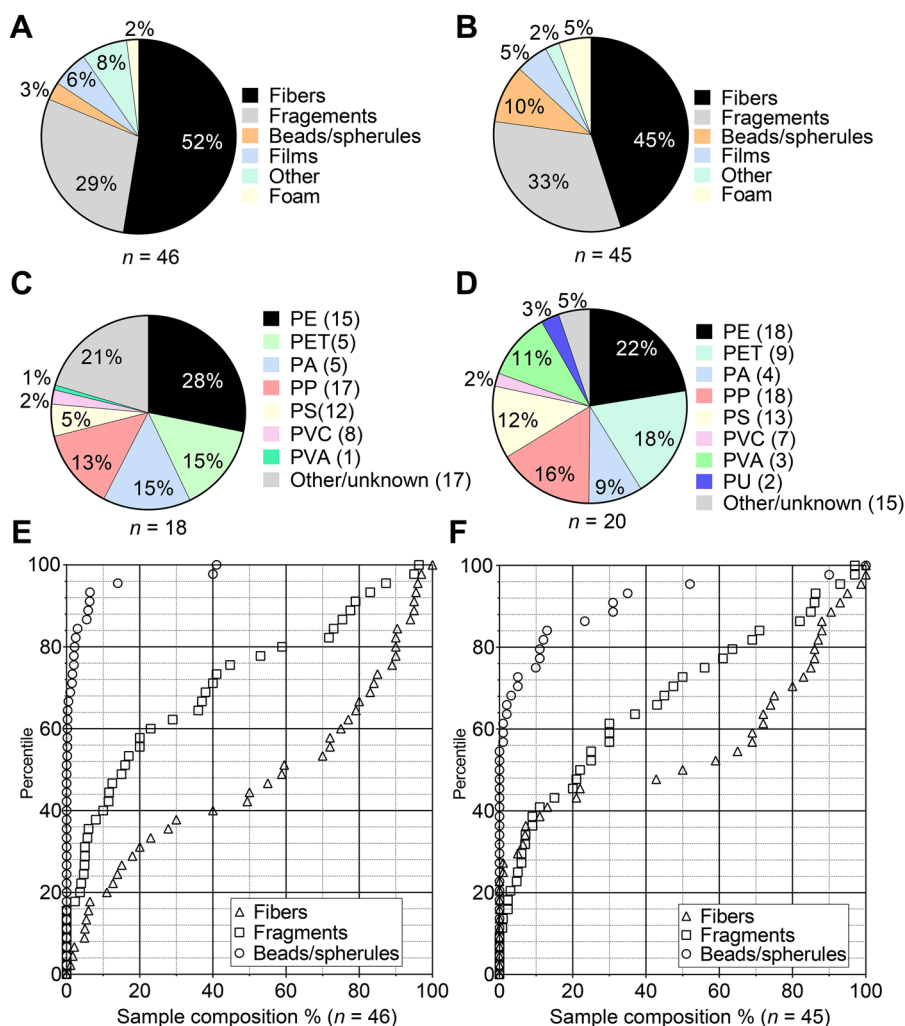
FIGURE 4: Measured environmental concentration sample summary characteristics. Average polymer composition per study in the water column (A) and sediment (B) and overall shape/morphology averages in the water column (C) and sediment (D). Sample percentages reported in reviewed studies of fibers, fragments, and beads were ranked and plotted to give 3 distributions reflecting sample shape morphology trends in the water column (E) and sediment (F). Studies were only included which intended to quantify all microplastic shape morphologies. Both freshwater and marine studies were included. PA = polyamide; PE = polyethylene; PET = polyethylene terephthalate; PP = polypropylene; PS = polystyrene; PU = polyurethane; PVA = polyvinyl alcohol; PVC = polyvinyl chloride.

tank or associate with flocculants and subsequently be removed (Gouin et al. 2015; Carr et al. 2016). In either case, it is unlikely that a large fraction of the microplastic load will remain in the aqueous phase of the treatment process and subsequently be released with effluents to the environment. Volertsen and Hansen (2017) estimated that WWTP effluent contributes only

3% of the total microplastic load reaching the environment. In addition, a recent fate modeling exercise predicted that effluent receiving rivers will efficiently retain many microplastics prior to reaching the ocean, including the most dominant of the microplastic size fractions found in WWTP effluent (Besseling et al. 2017), suggesting that freshwater sediments are the most

TABLE 1: Summary of wastewater treatment plant removals and effluent composition for specific treatment types reported in the literature[a]

| Treatment type | Reported removal | Effluent composition | | | Reference[b] |
		Fiber	Fragment	Bead/spherule	
Primary	50, 78%	Mainly Fibers			1, 2
Secondary	98, 99, 96%	36–48%	46–67%	0–9%	1, 3, 4
Tertiary	98, 97, 90, 99.9%	8.8%	91%	Not reported	2, 4–6
Membrane bioreactor	72, 99%	61–84%	11–33%	0%	4, 7

[a]Full table references are reported in the Supplemental Data.
[b]References are as follows: 1 = Murphy et al. (2016); 2 = Talvitie et al. (2015); 3 = Magnusson and Norén (2014); 4 = Michielssen et al. (2016); 5 = Ziajahromi et al. (2017b); 6 = Carr et al. (2016); 7 = Leslie et al. (2017).

relevant compartment when considering exposure to microplastics released through WWTP effluent discharge to rivers.

The available data indicate that a significant proportion of microplastics will be removed in WWTPs and, of those emitted in effluent, only a small proportion will be microbeads. Results thus far (i.e., removals) indicate that a far greater fraction of microplastics entering wastewater will be directed to sewage sludge instead of effluent. This suggests that spreading sewage sludge for agricultural applications may be a more pertinent exposure pathway to explore for microplastics released to wastewater systems (Nizzetto et al. 2016).

Are microplastics ingested by organisms?

Several field studies have documented the ingestion of microplastics in many species from multiple trophic levels and geographic areas (Table 2). We direct the interested reader to Lusher (2015) for an extensive review of animal ingestion of microplastics in the field. Microplastics have been detected in fish, invertebrates, and avian species (Table 2). Consistent with water and sediment microplastic occurrence data, the greatest proportion of microplastics detected in tissues is made up of fibers and fragments, with only a small proportion being beads. A recent study of 400 fish from the North Sea, employing strict quality control criteria, yielded only 2 microplastics in a single fish (Hermsen et al. 2017). Furthermore, fish and plankton sampled over the past 30 yr in the Baltic Sea showed no significant increases of internal microplastic concentration over time. Approximately 20% of the fish sampled contained microplastics, and 93% of these microplastics were fibers (Beer et al. 2017). Fiber abundance could be higher for 2 reasons: internal organism concentrations reflect aquatic and sediment MEC sample composition or fibers are not egested as efficiently as harder particles (Murray and Cowie 2011). Fish tend to have the lowest internal concentration, which may be attributable to reduced exposure (e.g., feeding strategy; Wagner et al. 2014); however, field studies have demonstrated that higher internal microplastic concentrations were correlated with higher surrounding microplastic concentrations and not

related to feeding mode, length, or weight for both deep water invertebrates and fish species (Courtene-Jones et al. 2017; Pazos et al. 2017; Steer et al. 2017). This connection was possible to establish because the authors also quantified microplastics in the surrounding water. This is not common practice but greatly aids in the interpretation of results. Therefore, field uptake studies can be improved by reporting microplastic concentrations both internally and externally.

In the laboratory, many studies have demonstrated uptake of microplastics into organisms. Scherer et al. (2017) found that microplastics co-exposed with algae significantly reduced microplastic ingestion by *Daphnia magna*, which is similar to a previous conclusion drawn by Ayukai (1987), where *Acartia clausi* demonstrated preferential feeding when exposed to algae and microplastic spheres. Weber et al. (2018) found that the microplastic body burden of *Gammarus pulex* depended on dose and age, whereas experiments conducted by Marń-Magán and Cañavate (1995) linked preferential ingestion to life stage in *Penaeus japonicus*. When quantifying microplastic ingestion rates, it is important to consider test conditions because the presence of food or the type of food could impact results, in addition to the feeding mode and life stage of the test species (Connors et al. 2017).

The ingestion of microplastics needs to be considered concomitantly with egestion rates to provide meaningful interpretation of the presence of microplastics in organisms. Laboratory microplastic exposure studies on fish and invertebrate species are numerous; however, few examine the question of whether microplastic ingestion affects egestion rates, particularly at concentrations similar to those found in the environment (Chua et al. 2014; Au et al. 2015; Scherer et al. 2017). There is evidence of efficient gut clearance in goldfish of both bead-shaped microplastics and fibers (Grigorakis et al. 2017). Furthermore, Mazurais et al. (2015) observed complete egestion of bead-shaped microplastics (10–45 μm) from *Dicentrarchus labrax* larvae after a 48-h depuration period. Significant microplastic egestion has also been demonstrated in invertebrates, despite concern that egestion could be impeded by their smaller size. Irregular particles (11–700 μm) were egested within

TABLE 2: Average and internal concentration range as well as microplastic sample composition reported in reviewed studies from the literature[a]

Trophic group	Concentration range (mean)	Sample composition % mean (range)		Reference[b]
Fish	0–19 (1.4) items/fish	38% (0–100)	Fiber	1–17
		27% (0–94)	Fragment	
		2% (0–24)	Bead	
Invertebrate	0.47–11.2 (2.8) items/organism	91% (65–100)	Fiber	15–24
	0.36–11 (3.05) particles/g	13% (0–13)	Fragment	
		5.3%	Bead	
Bird	14.2 items/bird	74% (55–100)	Fiber	25, 26
		7.7% (0–7.7)	Fragment	
		0%	Bead	

[a]Full references are reported in the Supplemental Data.
[b]References are as follows: 1 = Bellas et al. (2016); 2 = Silva-Cavalcanti et al. (2017); 3 = Rochman et al. (2015) 4 = Nadal et al. (2016); 5 = Neves et al. (2015); 6 = McGoran et al. (2017); 7 = Tanaka and Takada (2016); 8 = Wesch et al. (2016); 9 = Güven et al. (2017); 10 = Boerger et al. (2010); 11 = Davison and Asch (2011); 12 = Ory et al. (2017); 13 = Collignon et al. (2014); 14 = Alomar and Deudero (2017); 15 = Jabeen et al. (2017); 16 = Rummel et al. (2016); 17 = Pazos et al. (2017); 18 = Li et al. (2016); 19 = Davidson and Dudas (2016); 20 = Remy et al. (2015); 21 = De Witte et al. (2014); 22 = Leslie et al. (2017); 23 = Van Cauwenberghe and Janssen (2014); 24 = Courtene-Jones et al. (2017); 25 = Zhao et al. (2016); 26 = Amelineau et al. (2016).

36 h by *Allorchestes compressa* (Chua et al. 2014), and complete egestion of fibers was observed in 4 h by *Gammarus fossarum* (Blarer and Burkhardt-Holm, 2016); efficient gut clearance of beads and fragments (10–106 μm) by *D. magna* within 24 h, though fragments were slower to egest than beads (Frydkjaer et al. 2017); and complete egestion of a mixture of beads, fibers, and fragments ingested by *Idotea emarginata* (Hämer et al. 2014). Au et al. (2015) reported slower egestion of fibers than bead-shaped microplastics (which was equivalent to food egestion) in *Hyalella azteca*; however, complete egestion did occur in both exposures. Finally, field observations of Atlantic cod identified that the vast majority of stomachs found with microplastics were also full of organic content (Bråte et al. 2016). The authors proposed that microplastic gut clearance was therefore similar to food. These findings suggest that microplastic egestion will be significant in both fish and invertebrates and may be influenced by species and microplastic morphology; this information is important from a risk-assessment point of view and should be reported with all microplastic exposure studies.

Although many studies suggest that microplastic egestion is significant, there are also a few observations of particles translocating from the digestive tract. For example, *D. magna* exposed to 1-μm spheres exhibited translocation across the gut epithelial barrier (Rosenkranz et al. 2009). Crabs exposed to 0.5-μm spheres also demonstrated translocation to the hemolymph, gills, and ovary (Farrell and Nelson 2013). Tissue translocation of microplastics from the gut to the circulatory system has also been demonstrated in mussels exposed to <10-μm particles (Browne et al. 2008); however, repetition of this experiment in Pacific oyster did not result in translocation (Sussarellu et al. 2016). Von Moos et al. (2012) provided evidence of microplastic (<80 μm) uptake into the digestive gland of blue mussels, causing an inflammatory response at the cellular level. Lu et al. (2016) exposed zebra fish to 20- and 5-μm as well as 70-nm microplastics and found 5-μm and 70-nm particles in the gills, liver, and gut, whereas 20-μm particles were found only in the gills and gut. The mechanisms for translocation from the gut to the circulatory system and then to the liver are not well understood. The translocation of particles 5 to 150 μm is thought to be attributable to persorption, a phenomenon which occurs in vertebrate species where particles passively and infrequently pass from the gut to the circulatory system after ingestion (Volkheimer 1977). Interestingly, particles greater than the 150-μm size limit (which is the persorption threshold associated with humans) have been found in the fish liver (up to 600 μm; Avio et al. 2015). It may be possible, albeit unlikely, that the persorption threshold in fish is higher, allowing >150-μm particles to infrequently pass into the circulatory system (Jovanović 2017; Jovanović et al. 2018), or another currently unknown mechanism could be occurring. Collard et al. (2017) reported translocation of mainly 323-μm microplastics in anchovies and suggested 2 possible translocation theories: 1) the agglomeration of smaller pieces that were taken up, or 2) passage through the intestinal barrier. However, the authors state that methodological limitations prevent the precise localization of microplastics. There is also the possibility that studies demonstrating translocation of particles >150 μm could

be subject to contamination because follow-up research to define a possible mechanism for this translocation has yet to be undertaken (Avio et al. 2015; Jovanović et al. 2018). What is known is that translocation can occur and seems to be size-dependent, but is not consistently observed after every exposure. Particles <5 μm can enter the circulatory system more easily (e.g., nanoplastics), but smaller particles can also be removed more easily than larger particles (Jovanović 2017). It should be highlighted that methodological limitations and small study sizes prevent the precise localization of microplastics, making robust conclusions difficult to draw; furthermore, these studies, although useful, do not provide advancement toward understanding the mechanisms behind translocation. Thus, the mechanism behind the translocation of various particle sizes from the gut to the circulatory system and liver and the frequency of these events are important knowledge gaps that need to be addressed. With a better understanding of the relationship between translocation mechanisms, frequency, and particle size, evaluation of the risks that microplastic translocation may pose will become possible.

Similar to WWTP samples, analytical confirmation of the presence of microplastics presents significant challenges in the tissues of organisms (Vandermeersch et al. 2015; Hermsen et al. 2017), and caution should be exercised when interpreting results from studies only using visual identification methods (Rochman et al. 2015; Bellas et al. 2016; Davidson and Dudas 2016; Zhao et al. 2016; Silva-Cavalcanti et al. 2017). Close attention should also be paid to sample extraction and digestion methods because some are inefficient, potentially degrade, or color plastics in a sample, such as methods using nitric acid (Dehaut et al. 2016).

Trophic transfer of microplastics

The trophic transfer of microplastics has been suggested as an important biomagnification pathway for predators owing to their similarity to prey and small size, resulting in availability to lower trophic organisms (Andrady 2011). This could both impede feeding and permit microplastics to be passed to predators, which, after prolonged periods of feeding, may result in biomagnification (Wright et al. 2013). Trophic transfer of microplastics has been demonstrated in the laboratory (Farrell and Nelson 2013; Setälä et al. 2014; Tosetto et al. 2017); however, the circumstances of these conclusions are important to consider. Firstly, in these studies invertebrates have been limited to a diet of only microplastics, which could influence uptake (Scherer et al. 2017); secondly, invertebrates are then fed to predators prior to a depuration period; and thirdly, microplastic occurrence in predators is quantified prior to depuration, despite the high microplastic egestion rates reported in the literature for species in both trophic levels. It is important to note that these artificial conditions are poorly representative of environmental conditions and thus results should be interpreted with caution. The trophic transfer of microplastics has yet to be shown in the field, although a recent study reported that neither fish mass nor trophic level was related to microplastic ingestion, leading the authors to conclude that observed microplastic

presence is ephemeral, suggesting low biomagnification potential because of significant gut clearance (Güven et al. 2017). This agrees with laboratory studies demonstrating low microplastic gut retention times in fish (Mazurais et al. 2015; Grigorakis et al. 2017) and invertebrates (Ugolini et al. 2013; Hämer et al. 2014; Blarer and Burkhardt-Holm 2016), providing further evidence that accumulation will be minimal; however, available data do demonstrate that microplastics can be taken up by organisms in the environment.

DO MICROPLASTICS AFFECT MARINE AND FRESHWATER ORGANISMS?

Effect studies with microplastics have explored a range of endpoints including survival, growth, reproduction, moulting, and biochemical endpoints. In the present section we review the types of tests that have been employed and the results obtained.

Study conditions

A variety of experimental designs have been used to evaluate the impacts of microplastics on freshwater and marine organisms. The most common test material is polystyrene, despite polyethylene being reported as the most common polymer in environmental samples (Figures 4A and B and 5). The majority of studies (95%) have worked with smaller particle sizes than those that can be confidently detected in the environment (e.g., <131 μm; Figure 5). The majority of studies focus on spherical particles, with only a handful testing fibers (Au et al. 2015) or fragments (Imhof and Laforsch 2016), despite the prevalence of fragments and fibers in environmental samples, an issue also identified by a recent review on the subject (Phuong et al. 2016).

FIGURE 5: Summary of the test characteristics (particle types and sizes and test species) used in the identified effects studies for microplastics. Pie charts are presented for exposure particle size and polymer as well as test species trophic level. Test species are initially reported by trophic level, followed by the percentage of those studies that used either marine, freshwater, or terrestrial species. PA = polyamide; PE = polyethylene; PET = polyethylene terephthalate; PS = polystyrene; PVC = polyvinyl chloride.

The majority of test species used in the studies are from the primary consumer group (e.g., invertebrates), which is expected for ethical reasons (Figure 5), with the majority of studies investigating effects of microplastic exposure on marine organisms, suggesting a data gap for ecotoxicity pertaining to freshwater and terrestrial species.

Distribution of ecotoxicity endpoints

In Figure 6, NOECs and LOECs in terms of particles per liter from each of the ecotoxicity studies reviewed are presented. The endpoints have been separated according to the particle size ranges studied because this is thought to impact the likelihood of ingestion and therefore the effect (Jeong et al. 2016). Immediately it is clear that the particle sizes tested are much smaller than those that have been documented with confidence as occurring in the natural environment. Micro- and nano-particles are able to be studied in laboratory-based effects studies because they can be labeled to ease analytical detection, for example, with a fluorescent label (Kaposi et al. 2014). In most of the studies, spherical particles that were either precleaned or obtained straight from the manufacturer were used, whereas only 5 studies tested the effects of exposure to fibers (Hämer et al. 2014; Au et al. 2015) and weathered fragments (Rochman et al. 2013b; Imhof and Laforsch 2016; Ogonowski et al. 2016).

The ecotoxicity endpoint distributions (Figure 6) give a broad overview of our current understanding of the potential effects of microplastics. They include nonstandard and standard end-points from both acute and chronic tests, regardless of whether or not the test followed established guidelines such as those recommended by the Organisation for Economic Co-operation and Development (OECD). The majority of tests have resulted in a NOEC; however, in many cases this refers to the highest exposure concentration tested (Browne et al. 2008; Blarer and Burkhardt-Holm 2016; Watts et al. 2016; Chen et al. 2017). This would indicate that the true NOEC could actually be greater. Fragments are thought to have a higher potential to cause internal abrasion because of jagged or sharp edges; however, there are limited experimental data to confirm this. A single study thus far has reported a fragment effect concentration for 50% of the studied population (EC50), 8.6×10^7 particles/L for *D. magna* (Ogonowski et al. 2016). The tested particle size was approximately 1 mm, which is a relevant size in terms of reported microplastic MECs; however, the EC50 is orders of magnitude greater than the maximal MEC (e.g., 16.7–100 particles/L). Lethal doses for 50% of the tested population have also been reported for fibers, 71 430 fibers/L for the amphipod *H. azteca* (Au et al. 2015) and 13 000 fibers/L for the zooplankton *Ceriodaphnia dubia* (Ziajahromi et al. 2017a), which again is an order of magnitude greater than the highest reported MECs.

Endpoints presented in Figure 6 only pertain to reviewed studies where either particles per liter was reported or a conversion from mass per liter using particle size and density according to the methodology of Connors et al. (2017) was possible. In several cases, studies reported the exposure as mass or percentage of diet and without the necessary particle characteristics to enable a particle per liter conversion (i.e.,
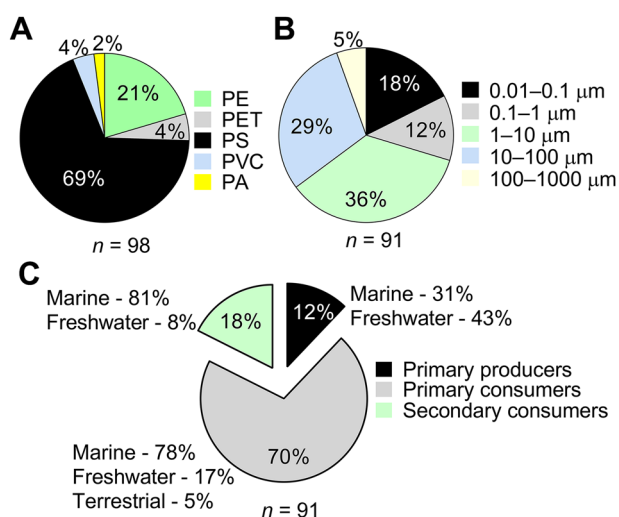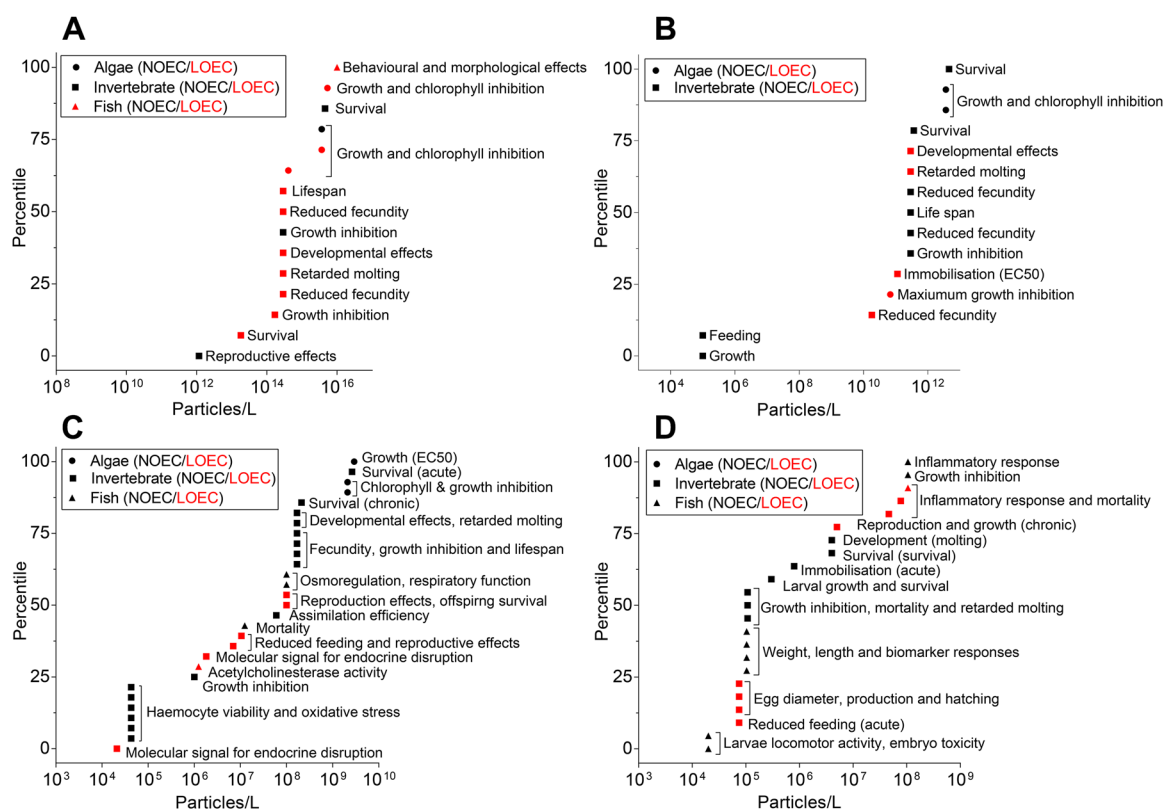
FIGURE 6: Microplastic cumulative ecotoxicity endpoint distributions for tests using particle sizes of 0.01 to 0.1 μm (A), 0.1 to 1 μm (B), 1 to 10 μm (C), and >10 μm (D). Red and black symbols represent lowest-observed-effect concentrations (LOECs) and no-observed-effect concentrations (NOECs), respectively. A cumulative distribution can be interpreted as where along the X-axis a NOEC/LOEC is likely to fall. For example in (A) the 25th percentile of LOECs/NOECs is approximately 10^{14} particles/L, whereas the 75th is approximately 10^{15} particles/L. Endpoints include a range of acute, sublethal, and standard and nonstandard endpoints identified by the present review (see Supplemental Data for references). EC50 = median effect concentration.

Cedervall et al. 2012; Hämer et al. 2014; Imhof and Laforsch 2016; Tosetto et al. 2016; Lwanga et al. 2017). Several studies have tested multiple particle sizes but based the exposure on mass per liter; therefore, smaller microplastic sizes had particle per liter counts orders of magnitude greater than the larger particle sizes tested (Jeong et al. 2017, 2016; Lu et al. 2016; Chen et al. 2017). In these cases, it is not possible to evaluate whether smaller particles sizes are more harmful than larger particle sizes. Reporting in particles per liter is preferable because it is directly comparable with environmental occurrence data and a better option to encompass the diversity of microplastic particle sizes.

Effects from molecular or biomarker endpoints can be difficult to scale up to effects in the environment; however, we report these endpoints in the interest of canvasing the breadth of reported effects to date. Unfortunately, not all studies could be included, for example, Rochman et al. (2013b). Important biomarker responses related potentially to lack of nutrition were reported; however, a conversion to particles per liter was not possible because the authors did not report the size distribution of microplastics used in the study. In addition, the study, similar to others (Paul-Pont et al. 2016), lacked a negative control. For example, part of the diet was replaced by plastic; therefore, effects seen in treatment fish could be attributable to reduced diet, not the addition of the plastic

(Duis and Coors 2016). A more realistic approach would be the addition of plastic to food without replacement (Imhof and Laforsch 2016) or including a negative control (Karami et al. 2016; Watts et al. 2016). A similar issue is observed in invertebrate studies where effects are attributed to microplastic intake without consideration of effects experienced from a lack of, or an inappropriate, food source (Huntley et al. 1987; Scherer et al. 2017).

The usefulness of the ecotoxicity testing strategies employed in many of the studies, in terms of environmental relevance, has been questioned (Phuong et al. 2016). Study design limitations include the lack of environmental relevance pertaining to the size, shape, and concentration of tested microplastics; lack of detailed test particle characterization such as the size distribution, density, and assessment of chemicals potentially already sorbed prior to exposure (Connors et al. 2017); variability in reporting units (e.g., mass per liter or particles per liter, percentage of diet); the use of nonstandard endpoints or biomarkers (Karami 2017); and lack of appropriate controls (e.g., negative controls; Duis and Coors 2016). In conclusion, data from laboratory-based studies indicate that some microplastics have the potential to adversely affect organisms when exposed at very high concentrations (e.g., EC50 of 8.6×10^7 particles/L; Ogonowski et al. 2016). However, there is a mismatch between the size,

morphology, and concentration of microplastics investigated in the effects studies and those monitored in the environment. Furthermore, environmental microplastics exist as a mixture, and this should be reflected in ecotoxicity studies; for example, testing fibers, fragments, and beads simultaneously in the appropriate proportions would be useful (Hämer et al. 2014; Ziajahromi et al. 2017a), in addition to investigating lesser studied particle types, such as films, fibers, and fragments because evidence suggests that these morphologies could be more harmful than beads (Gray and Weinstein 2017; Hodson et al. 2017; Ziajahromi et al. 2017a). As a result, there are significant data gaps pertaining to microplastic ecotoxicity (Phuong et al. 2016; Connors et al. 2017; Karami 2017), and standardized testing which can generate EC50 data would be useful for regulatory risk assessment. Study designs should incorporate adequate controls and follow, when appropriate, OECD test guidelines. Most importantly, there is an urgent need for both monitoring and effect studies to report in concentrations that are comparable. In the field of particle toxicology, units of particle number per volume, surface area per volume, and mass per volume have been used. In performing these studies, it may be appropriate to use a number of standardized units. The key is that authors fully characterize the test particles used in ecotoxicity studies and report these data to enable conversions between the various units, which allows comparison to exposure data.

Do microplastics act as vectors of persistent organic pollutants directly and through food chains?

It has been claimed that, because of their physicochemical properties, microplastics adsorb significant loads of hydrophobic organic contaminants (HOCs) and that when these microplastics are ingested, they can act as a vector for the transport of HOCs into the organism (Cole et al. 2011; Wright et al. 2013). This is sometimes referred to as the "Trojan horse effect." We therefore examined literature that has discussed the potential for microplastics to act as vectors of HOCs to 1) identify the most influential papers cited as evidence of this phenomenon, and 2) determine whether the influential studies do indeed provide evidence of the Trojan horse effect.

Plastic is efficient at sorbing HOCs, mainly because of its hydrophobicity; and this been demonstrated in both the laboratory (Bakir et al. 2012, 2014b) and the field (Mato et al. 2001; Rios et al. 2010). The amount of and the particular HOCs adsorbed will be dependent on the polymer type and available surface area (Rochman et al. 2013a). The amount of time HOCs take to reach an equilibrium between the plastic and the surrounding environment has been shown to take months to years (Endo et al. 2013; Rochman et al. 2013a; Koelmans et al. 2016), whereas desorption half-lives for some compounds range from 14 d to hundreds of years (Endo et al. 2013). This, in conjunction with recent modeling evidence (Gouin et al. 2011; Bakir et al. 2016; Koelmans et al. 2016; Lee et al. 2017), has led many to conclude that microplastics in the environment are expected to act as sinks for HOCs and not sources to organisms postingestion (Herzke et al. 2016; Kwon et al. 2017). Conversely, it has been suggested that internal gut conditions will facilitate HOC desorption (Teuten et al. 2007; Bakir et al. 2014a), and many studies published in 2017 suggest that this contaminant exposure pathway is highly relevant, indicating that the debate is still ongoing.

It is difficult to test the Trojan horse effect, and studies that have attempted it have almost exclusively been limited to laboratory experiments. Modeling studies have also been employed to determine whether the effect is possible based on theory. An analysis of the different studies that have explored the effects of ingested microplastics on HOC uptake is provided in Table 3. Correlations of HOCs in wild species with environmental microplastics (Ryan et al. 1988; Tanaka et al. 2013) provide little proof that plastics are responsible for observed contamination of organisms. Laboratory studies that have employed environmentally unrealistic test gradients using either clean exposure media (sand or water), clean animals, or unrealistically high HOC concentrations also only provide limited proof of the effect (Ziccardi et al. 2016). It is not surprising that a transfer under these laboratory conditions can be shown (Browne et al. 2008; Chua et al. 2014; Wardrop et al. 2016); however, these results need to be put into an environmental context. For example, several authors have observed less transfer from plastics than other more abundant and naturally occurring particles (e.g., sediment), suggesting that the transfer of contaminants from plastic is not significant (Browne et al. 2008; Beckingham and Ghosh 2017). Furthermore, studies with the polycyclic aromatic hydrocarbon phenanthrene indicate that greater sorption occurs to plankton than to plastic, suggesting that normal food sources may be a more important uptake pathway for certain HOCs than plastic (Frydkjaer et al. 2017). Another important component to consider is the desorption half-life from plastic. Several laboratory studies have reported complete egestion of microplastics (in unrealistically high exposures) in 24 to 48 h (Grigorakis et al. 2017). This, in addition to the low internal concentrations of microplastics in wild animals (Table 2), would suggest that plastic does not accumulate in the gut long enough to facilitate desorption, even if gut surfactants did slightly enhance the thermodynamic favorability of HOC desorption.

To demonstrate the inconclusive categorization for studies seemingly providing evidence of the Trojan horse hypothesis, we use a study where *Oryzias latipes* were exposed to microplastics associated with a concentration of HOCs measured in the marine environment (Rochman et al. 2013b). Fish were kept in clean water that was refreshed regularly, with contaminated plastics sprinkled into the water with food (Rochman et al. 2013b). This study design is not actually testing the Trojan horse hypothesis because it is impossible to differentiate whether microplastics were ingested and HOCs subsequently desorbed internally or whether the unrealistic gradient between the clean water and microplastics sorbed with HOCs caused the HOCs to leach directly into the water and subsequently associate with the fish (i.e., bioconcentration instead of bioaccumulation).

TABLE 3: Evaluation of evidence for ingestion and subsequent desorption of contaminants from microplastics as a significant exposure pathway[a]

| Study type | Evidence category | | | Reasoning | Reference |
	Demonstrated	Inconclusive	Not supported		
Field			✔	Correlation between PCBs and mass of ingested plastic, correlation ≠ causation.	Ryan et al. (1988)
Field			✔	High degree of PCB and other contaminant absorption to polyethylene in seawater.	Mato et al. (2001)
Model / laboratory		✔		Presence of plastic will increase sediment organism exposure, observed enhanced desorption rates in synthetic gut surfactant; model limited: no biofouling or transport from organics for comparison.	Teuten et al. (2007)
Laboratory		✔		Chicks fed resin pellets, total PCB load not significant, but lower chlorinated congeners significantly different; small sample size and large variability among replicates.	Teuten et al. (2009)
Model			✔	"MP as a vector for PBT substances may be relatively small compared to other exposure pathways."	Gouin et al. (2011)
Field		✔		PBDE composition found in seabirds similar to plastic in stomach, prey samples taken 7 yr later, >1000 km away did not contain similar PBDEs.	Tanaka et al. (2013)
Laboratory			✔	Transfer from plastic demonstrated to worms, determined impact of plastic on PCB transfer small.	Besseling et al. (2013)
Laboratory			✔	Transfer demonstrated (high plastic, contaminant concentration) but 250% less than transferred from sediment (lower concentration than plastic).	Browne et al. (2013)
Laboratory		✔		Experimental design cannot differentiate between desorption in water and subsequent uptake or via internal gut releases (Trojan horse); unrealistic contaminant gradient between pellets and exposure water.	Rochman et al. (2013b)
Laboratory			✔	Significance at 10 times environmentally relevant concentrations; at environmentally relevant concentrations, uptake into amphipods was less than sediment.	Chua et al. (2014)
Model			✔	Microplastic could be a substantial exposure pathway to worms; however, conditions required unlikely in environment; pathway for fish appears negligible.	Koelmans et al. (2014)
Field/model			✔	POP concentration in seabirds not correlated with plastic ingestion; modeling suggests more likely to act as passive sampler.	Herzke et al. (2016)
Laboratory			✔	Demonstrated uptake in worms; however, plastic 76% less than sediment; concluded transfer dominated by natural particles.	Beckingham and Ghosh (2017)
Model			✔	Modeled existing empirical data, flux of HOCs bioaccumulated from natural prey > flux from plastic.	Koelmans et al. (2016)
Model			✔	Plastic is not a quantitatively important pathway for transfer of adsorbed chemicals.	Bakir et al. (2016)
Model			✔	Role of plastic as a vector to transfer to organisms minimal (PAHs, fugacity).	Lee et al. (2017)
Laboratory			✔	No elevation from sedimented microplastics to larval fish in unrealistically high exposures.	Sleight et al. (2017)
Laboratory			✔	Ingestion of microplastics is unlikely to increase worm exposure to zinc.	Hodson et al. (2017)

[a]Studies conducted prior to 2016 are most commonly cited as evidence/support for the phenomenon.
HOC = hydrophobic organic contaminant; MP = microplastic; PBDE = polybrominated diphenyl ether; PBT = persistent, bioaccumulative, toxic; PCB = polychlorinated biphenyl; POP = persistent organic pollutant.

A mass balance calculation was undertaken to determine the theoretical maximum concentration that the HOC-associated, microplastic-exposed fish could have based on the reported concentrations on the pellets (Figure 7). Contamination of the control fish with HOCs is evident (Figure 7) and may be a result of the use of cod oil in the diet (Rochman et al. 2013b). To demonstrate the transfer of HOCs from plastic, the reported concentrations (black dots) would need to fall somewhere along the blue bars; this only occurs for fluoranthrene, pyrene, PCB 123 and PCB 187, none of which were reported as significantly different between control and treatment fish. There could be many reasons why the experimental results do not match the
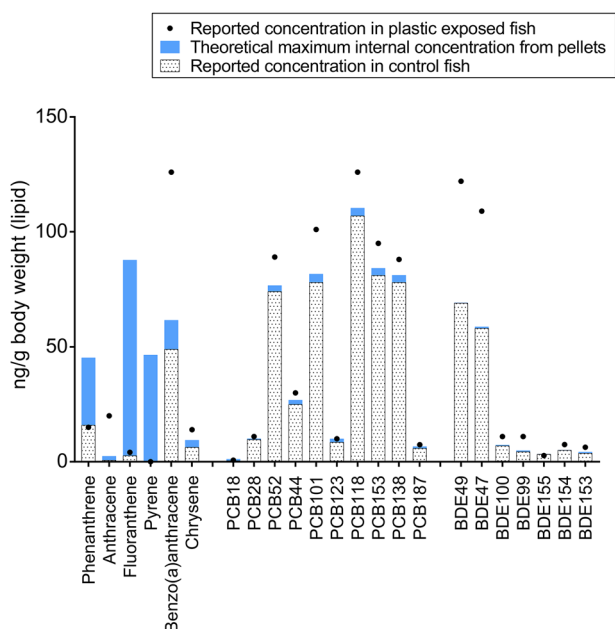
FIGURE 7: Calculated theoretical maximum lipid concentrations in marine plastic–exposed fish (blue bars) based on a mass balance analysis of reported initial marine pellet concentrations from Rochman et al. (2013b). Reported control fish (dotted bar) and marine plastic–exposed fish (black dots) lipid concentrations are also plotted. Fish were assumed to be 300 mg and the lipid content ranged from 2.1 to 6.2% (C. Rochman, University of Toronto, Toronto, Ontario, Canada personal communication).
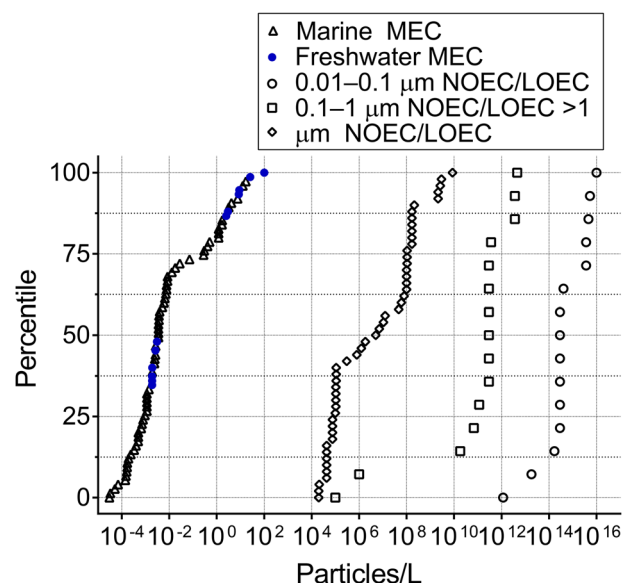

FIGURE 8: Cumulative species endpoint distribution plotted with the measured environmental concentration distribution (marine and freshwater). Three separate endpoint distributions are plotted which contain both no-observed-effect concentrations and lowest-observed-effect concentrations from acute and chronic tests from fish, invertebrates, and algae. Only endpoints related to growth, mortality, and reproduction are plotted. Ecotoxicity endpoints are divided into 3 distributions based on test particle size: 0.01 to 0.1 μm, 0.1 to 1 μm, and >1 μm. LOEC = lowest-observed-effect concentration; MEC = measured environmental concentration; NOEC = no-observed-effect concentration.

mass balance calculations. What we can say is that the added contaminant contribution from pellets in most cases is substantially less than what the control fish were exposed to. When pellet HOC concentrations were much greater than those found in the control fish concentrations (pyrene, phenanthrene, fluoranthene), a corresponding concentration spike in microplastic treatment fish was not observed, suggesting that the microplastics retained these compounds. Therefore, this study and those using similar experimental designs are inconclusive and cannot be used in support of the Trojan horse hypothesis. A better design, for example, would be to use marine fish and keep them in tanks of relevant seawater, then subsequently introduce the presorbed microplastics, as well as controls without microplastics in seawater and clean HOC-free water. In conclusion, the available evidence either does not support that microplastics can act as a vector of HOCs into organisms or is inconclusive. We were not able to find a study where uptake of HOCs could truly be attributed to transport into the organisms by microplastics.

DO MICROPLASTICS POSE A RISK TO THE ENVIRONMENT?

A major component missing thus far from microplastic environmental research is putting the effects and occurrence studies into the context of risk. In a word analysis of abstracts from all reviewed literature, "risk" was determined to be the 188th ranked word, whereas "concentration" and "effect" ranked 10th and 11th, respectively. Risk assessment provides a starting point for determining the particular microplastic

shapes, sizes, or polymers that are most likely to be harmful in the real world and to identify geographical regions at greatest risk. This information would help to focus future research efforts on microplastics of greatest concern and help inform which, if any, mitigation strategies should be introduced and where they should be introduced. Therefore, in the next sections, we bring together the results of the monitoring and the effects studies to determine whether, based on the current evidence base, there is a likelihood for negative impacts in the natural environment.

Comparison of MECs with effects endpoints

To put the data from the effects studies into context, we initially compare the MEC distributions with effect concentration distributions (Figure 8). This comparison is limited to effects endpoints pertaining to growth, mortality, and reproduction because these are the standard endpoints used in the regulatory risk assessment of chemicals (Connors et al. 2017). The lowest LOECs/NOECs (obtained for particles in the >1-μm size range) from the effect studies were more than 2 orders of magnitude greater than the highest MEC (Figure 8). Based on these data, there is therefore little evidence that concentrations of microplastics seen thus far in the environment have a negative effect on organisms, particularly given that many of the monitoring studies are thought to have overestimated concentrations because of limitations in the identification methodologies that we have described.

Species Sensitivity Distribution

Comparison of MECs with the ecotoxicity endpoint distributions is useful for gauging the overall trends between microplastic particle size–related effects and MECs; however, there are also enough data available in the literature to take the first steps toward creating SSDs for microplastics and performing a probabilistic assessment of risks. An SSD is a cumulative probability function based on ecotoxicity tests from multiple species representing a range of taxa (Posthuma et al. 2002). When these endpoints are combined into a distribution (lognormal), predictions of percentage of species affected can be made (Newman et al. 2000). Therefore, community-level risk can be estimated by extrapolating this statistical distribution from individual species toxicity (Garner et al. 2015). Different environments (e.g., freshwater, marine, and terrestrial) contain specialized species that employ a variety of feeding strategies (e.g., filter feeders) or life history characteristics that can increase microplastic exposure. Equally they could be particularly sensitive to microplastic ingestion because of body size or the inability to egest microplastics (Wright et al. 2013). The SSD captures this interspecies variability to a stressor (e.g., microplastics), which can then be used to derive key risk-assessment components such as predicted-no-effect concentrations (European Chemicals Bureau 2003) or a 5% hazard concentration (HC5). The HC5 is a key regulatory parameter used to derive legally binding environmental quality standards and translates to the concentration where 5% of species in an ecosystem would be harmed (Wheeler et al. 2002). Also, SSDs can be used to derive maximum acceptable concentrations from a limited set of laboratory data (Silva et al. 2014).

An SSD was built using the Species Sensitivity Distribution Generator (US Environmental Protection Agency 2014). There are several assumptions and criteria required to build a representative SSD (Posthuma et al. 2002), and the authors recognize that there are several limitations with the distribution presented in Figure 9. The usefulness of an SSD depends on the

data it is created from; therefore, an important caveat to consider for the SSD presented in Figure 9 is that both NOECs and LOECs were used so that a range of species ($n = 9$) could be included, covering key taxa (e.g., fish species, isopods, copepods echinoderms, and crustaceans; see Supplemental Data for references). Only mortality, reproduction, and growth endpoints (Connors et al. 2017) from the largest particle size class (10–5000 μm) of ecotoxicity studies were considered because this size fraction is most relevant to particle sizes measured in the environment and consequently most representative based on current approaches. It should be noted, however, that only a single ecotoxicity study where a particles per liter value could be calculated used a >100-μm particle size exposure. If no significant effect was reported or the concentration below the LOEC was reported, this was considered the NOEC, whereas LOECs were the lowest concentration that had a significant effect. Endpoints were included that may not have adhered to high-quality tests that are desirable for SSDs (Wheeler et al. 2002). Marine and freshwater data were combined in the SSD presented to increase statistical power because alone not enough data are yet available to build an SSD for the freshwater or marine environment singly. Freshwater- and marine-specific SSDs are presented in the Supplemental Data. We present the first attempt to build an SSD for the risk assessment of microplastics, which in itself cannot provide regulatory guidance; however, it provides a starting point for what the SSD will look like and should be updated as more relevant data become available.

The confidence intervals of the 95% MEC and the 5% effect concentration do not overlap; the HC5 is 6.4×10^4 particles/L, 3 orders of magnitude greater than the 95% MEC, 8.5 particles/L, which, based on current data, indicates that risks are limited. Knowledge gaps do, however, need to be addressed to improve the quality and relevance of the SSDs and enable sound probabilistic risk assessment of microplastics in the environment (Koelmans et al. 2017). This includes ecotoxicity testing of relevant particle size and shape fractions, standardized testing, improved reporting of methods and results, and a greater focus on freshwater and terrestrial compartments. We have provided a starting point to be refined as research progresses, which, despite the caveats, does likely provide a general idea of what a refined SSD will look like. The MEC distribution could begin to overlap with the SSD when methods to measure smaller particle sizes in the environment emerge. This would be useful for putting the vast majority of current ecotoxicity studies in an environmental context and should be considered a research priority. On the other hand, ecotoxicity data from the 10- to 5000-μm size fraction were nearest the concentrations reported in the environment (Figure 8).

Overall, the comparison of MECs with effects endpoints does not support the claim of some that microplastics are negatively impacting the health of organisms in the environment. Concentrations of microplastics seen to cause effects on organisms are orders of magnitude higher than concentrations of microplastics measured in the environment. There are several limitations to keep in mind with regard to this comparison. We know that approximately half of the reported MECs have fiber contents
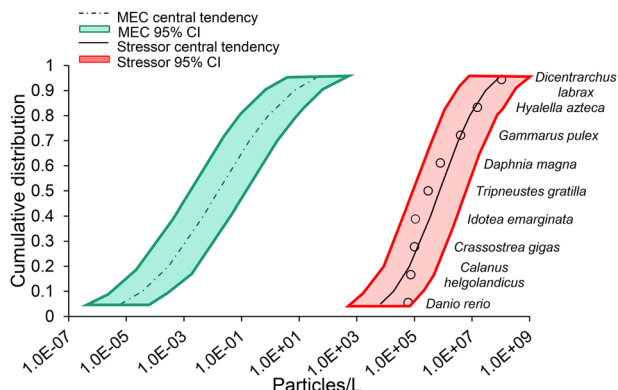

FIGURE 9: Species sensitivity distribution plotted with the 95% confidence interval (CI; red) based on no-observed-effect concentrations and lowest-observed-effect concentrations from studies of particles in the size range of 10 to 5000 μm (most relevant to environmental size distributions). The measured environmental concentration (MEC) cumulative distribution is also plotted (marine and freshwater MECs) with the 95% CI (green).

>50%, followed by fragments, neither of which are well represented in the effects studies, which tend to focus on beads/spheres (Hämer et al. 2014). The effects studies have also focused on particle sizes much smaller than those typically monitored in the environment. To answer the question of whether microplastics negatively impact organisms in the environment, the size range of microplastics needs to be clearly defined, monitoring studies need to characterize the complete size range of microplastics that occur in the environment, and effects studies need to work with test materials (plastic types, sizes, and shapes) that are consistent with those found in the environment. Only then will we be able to come to any conclusion as to whether microplastics negatively impact the environment or not.

CONCLUSIONS AND RECOMMENDATIONS

Microparticles do occur in the environment, but based on our analysis, there is currently limited evidence to suggest that they are causing significant adverse impacts or that they are increasing the uptake of hydrophobic organic compounds into organisms. This conclusion is in line with conclusions from others, calling into question the claims around risks posed by microplastics (Koelmans et al. 2017; Burton 2017). However, based on the current evidence, it is impossible to conclude that microplastics do or do not cause harm to the environment. This is attributable to the fact that monitoring efforts tend to focus on only a fraction of the microplastic size range that could occur in the environment and that effects studies tend to work with materials which are not the ones currently being monitored. Only limited data are available for freshwater environments, with even less for terrestrial systems, even though exposures in these environments could be greater than those in the marine environment. To determine whether microplastics cause harm in the environment, work therefore needs to focus on the following 3 aspects.

First, exposure of the environment to microplastics. Higher-quality occurrence data are needed in a broader range of compartments (i.e., including freshwater and terrestrial systems). This monitoring needs to determine concentrations of the complete size range of microplastics that occur in the environment. Concentrations need to be expressed in meaningful units that can be compared to effects study data. Accurate classification and chemical characterization of particles are essential. Monitoring of sources, such as diffuse (e.g., tire ware, paints, coatings) and point sources (e.g., industrial emissions and WWTPs) is needed to establish what the major sources are of microplastics in the environment. This will likely require the development of new sampling and analytical methodologies with lower concentration and size detection limits which are able to detect all microplastics and their transformation products, such as nanoplastics, in the natural environment. The lessons learned from other fields, such as nanoparticles, and interdisciplinary work involving analytical chemists and physicists could be valuable to help tackle these analytical challenges. The use of exposure modeling approaches, such as that used by Lambert et al. (2013) to characterize environmental exposure to latex and its degradation products, will also help to characterize real-world exposures. Exposure modeling may be particularly useful in situations where detection of a material is not possible because of limitations in current analytical methodologies and can provide information at greater spatial and temporal resolution than monitoring studies and help to identify major sources of exposure. To inform this exposure modeling, better information is needed on the types of macro- and microplastics in use, the amounts used, and the usage patterns, as well as information on the fate and behavior of these materials from laboratory and semi-field simulation studies.

Second, effects characterization. Effects studies are needed on the types of microplastics that actually occur in the environment and on their transformation products, such as nanoplastics. In particular, more work is needed on the effects of fragments and fibers of the size ranges currently being observed in the environment and on the effects of secondary microplastics. Studies need to characterize potential effects on not only marine organisms, but also freshwater and terrestrial species. Although studies should explore potential impacts on nonstandard organisms that could, because of their traits, be vulnerable to microplastic exposures, they should focus on ecologically relevant endpoints (e.g., mortality, growth, and reproduction) that are used in the assessment of risks of standard chemicals. For secondary microplastics, where the environment will likely be exposed to a complex mixture of particles of different sizes and shapes (Lambert et al. 2013), the use of semi-field environmental degradation studies on microplastics (e.g., Lambert and Wagner 2016) followed by effects testing on the resulting materials (e.g., Lambert et al. 2013) might help to determine whether these materials are causing harm or not.

Third, assessment of microplastic risks. The discussion around microplastics in the environment needs to be risk-based because occurrence does not always equate to impact and just because an effect is seen in the laboratory does not mean that the effect will occur in the real environment. Better design of monitoring and effect studies so that they yield data that inform risk assessment will mean that it will be possible to establish the degree of risk in different regions of the world and to identify activities and practices contributing most to the risk. This will mean that policies can be informed by sound science and that they will then actually have impact on the health of the environment.

We have presented the first detailed risk assessment of microplastics in the environment, using both a probabilistic method (SSD) and an ecotoxicity endpoint distribution to include nonstandard endpoints to demonstrate that current ecotoxicity is not comparable with MECs in terms of particle size; however, initial assessment provides little evidence of microplastics causing harm in the real environment. We have also demonstrated that significant evidence for microplastics acting as a vector for HOCs into organisms has yet to be proven and that recent laboratory and modeling evidence suggests that the impact of this exposure pathway is minimal. There is currently limited evidence to suggest that adverse environmental impacts are caused by microplastics; however, there are major knowledge gaps that urgently need to be addressed to confirm or disprove this.

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