In This Issue:

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Microplastics: Addressing Ecological Risk Through Lessons Learned

Kristian Syberg,*† Farhan R. Khan,† Henriette Selck,† Annemette Palmqvist,† Gary T. Banta,† Jennifer Daley,‡ Larissa Sano,§ and Melissa B. Duhaime‖

†Department of Environmental, Social and Spatial Change, Roskilde University, Roskilde, Denmark
‡School of Natural Resources and Environment, University of Michigan, Ann Arbor, Michigan, USA
§Cooperative Institute for Limnology and Ecosystems Research, University of Michigan, Ann Arbor, Michigan, USA
‖Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan, USA

Abstract—*Plastic litter is an environmental problem of great concern. Despite the magnitude of the plastic pollution in our water bodies, only limited scientific understanding is available about the risk to the environment, particularly for microplastics. The apparent magnitude of the problem calls for quickly developing sound scientific guidance on the ecological risks of microplastics. The authors suggest that future research into microplastics risks should be guided by lessons learned from the more advanced and better understood areas of (eco) toxicology of engineered nanoparticles and mixture toxicity. Relevant examples of advances in these two fields are provided to help accelerate the scientific learning curve within the relatively unexplored area of microplastics risk assessment. Finally, the authors advocate an expansion of the "vector effect" hypothesis with regard to microplastics risk to help focus research of microplastics environmental risk at different levels of biological and environmental organization.* Environ Toxicol Chem 2015;34:945–953. © 2015 SETAC

Keywords—Microplastics; Nanoparticles; Mixture toxicity; Vector effects; Ecological risk

Introduction

There is growing concern over the ecological risk of microplastics among regulators, the scientific community, and the public [1,2]. The use of plastics has gradually increased since the middle of the last century, and yearly production volumes now have surpassed 200 million tons [3]. This intensive use has led to a widespread distribution of plastics in the aquatic environment [4], a significant part of which is present as microplastics (plastic particles with a diameter <5 mm) [5]. Microplastic particles were first discovered and are best documented in the center of ocean convergences [6], where currents can concentrate them to levels of 500 000 particles/km^2, 10^2 to 10^4 times greater than outside these zones [7]. However, microplastics are now found worldwide in all aquatic compartments (surface water, water column, and sediments) [3], as well as in many aquatic animals, from invertebrates [8] to whales [9]. Studies in the Pacific Ocean have reported that more than 90% of the tows contained microplastics, with similar observations elsewhere [3]. A newly published study estimated that more than 5 trillion pieces of plastics are currently floating in the oceans [4]. Negative effects of microplastic exposure in benthic aquatic systems have been reported, including toxicity by reduced feeding activity and enhanced bioaccumulation of sorbed contaminants [10] and decreased energy reserves after consumption [8].

Microplastics can be grouped into primary and secondary materials (Figure 1), each with several subcategories. Primary microplastics are plastics produced in the micron size and most commonly used in facial cleansers and cosmetics or as air-blasting media for cleaning rust and paint off machinery and boat hulls. Secondary microplastics are microsized

* Address correspondence to ksyberg@ruc.dk.

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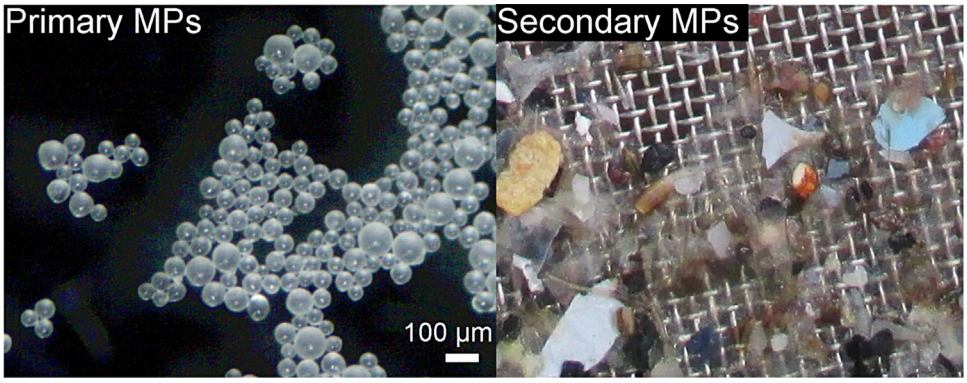


FIGURE 1: Primary and secondary microplastics (MPs). The primary microplastics or "microplastic beads" are produced in the micron size and used in cosmetic products, such as scrubs and exfoliants, and in industrial processing, such as sand blasting. Primary microplastics, such as the polyethylene beads (10–106 μm) pictured, are typically uniform in shape and composition. Secondary microplastics are micron-sized after the degradation of larger plastic debris. They are typically much more diverse in shape, size, color, and composition than primary microplastics, as can be seen in a sample trawled from the Mediterranean Sea.

fragments derived from the breakdown of larger plastic debris by processes such as biodegradation and photodegradation and physical wave-action [11]. Although it is not known yet which form dominates in the environment, the balance likely is location-dependent; primary microplastics might be more important in close proximity to wastewater effluents sites, whereas secondary microplastics may dominate in the open sea [4].

The heterogeneity (differences in polymer type, size, shape, color) of microplastics makes high-throughput quantification a challenge; thus, standard methods for sound exposure assessments are lacking [1]. The most important sizes and shapes with regard to ecological risk are unknown; however, previous findings suggest that characteristics such as form, size, age, and color can be important for the interaction of microplastics with contaminants and the accumulation in biota [3]. Microplastics can sorb a wide range of pollutants, possibly altering their bioavailability, fate, and flux into other environmental compartments [10]. Some plastics have toxic properties themselves (e.g., polyvinylchloride), whereas others contain additives to optimize their physical attributes, such as softeners that can leach into the environment. In addition, microplastics may cause physical impairment by adsorbing to filter appendages of invertebrates, thus affecting ventilation and feeding activity [8]. This suggests that microplastics may have both direct and indirect hazardous properties as a result of their chemical and physical characteristics.

Despite the paucity of information on ecological risks posed by microplastics in personal care products, some major corporations have pledged to phase out primary microplastics. In addition, regulations are being enacted that mandate phaseouts in coming years [12]. These eliminations eventually will reduce the number of microplastics entering the environment. However, the high amounts of recalcitrant microplastics currently in the environment, the fact that other microplastics will continue to be produced, and the fact that large plastic debris in the environment breaks down to secondary microplastics will lead to continued ecological exposures in the future. This reemphasizes the need to increase our understanding of the fate and ecological effects of microplastics in the aquatic environment.

Science is lacking to support evidence-based decision making with respect to ecological exposure to, effects of, and risks posed by microplastics. There is a critical need to better understand the range of likely exposures, their temporal and spatial variability, and the likely ecological receptors to determine the potential for adverse effects. Many of these concerns parallel those associated with (eco) toxicology of engineered nanoparticles (ENPs) and chemical mixtures. Exploration of how risk is assessed in these 2 areas can help guide the development of hypotheses in the area of microplastics risk. A key question in the risk assessment of ENPs relates to particle characterization. Similar to microplastics, ENPs have a diversity of particle characteristics that affect the likelihood of both uptake and target organ effects. Furthermore, ENPs are synthesized with different coatings and, similarly, microplastics have a range of sorption properties associated with their varying additives [13]. These characteristics unique to different microplastics are important to consider when addressing their environmental impact, because they will influence their fate and result in varying degrees of chemical sorption to the microplastics from ambient waters, wastewaters, and sediments where they reside. The study of the combined toxicological effects of microplastics and other contaminants together with possible physical impairment of particle ingestion have analogies to approaches used for assessing the risks of chemical mixtures (i.e., multiple stressors potentially with different modes of action). When addressing risk from chemical mixtures, the aim is to quantify the combined effects of more than 1 stressor and to assess whether interactions between the stressors involved causes mixture-specific effects that deviate from additivity (i.e., synergy or antagonism).

Toward Better Characterization of Ecological Risk of Microplastics—Lessons from ENPs and Mixtures

To conduct risk assessments of microplastics in the environment, both exposures and effects must be quantified properly. This requires a better understanding of properties of microplastics and their environmental fate, importance of interaction with various biological receptors, and mechanisms of toxicological action leading to potential effects of microplastics. In the following sections we highlight current understandings and point toward future challenges related to 4 topics: quantifying environmental exposure to microplastics, properties of microplastics and their environmental fate, importance of interaction with biological receptors, and mechanisms of toxicological action leading to potential negative effects. Parallels to ENPs and mixtures are made to suggest possible focuses for future research.

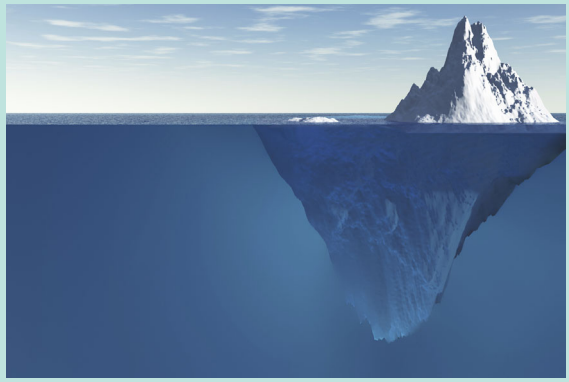
Quantifying environmental exposure to microplastics

For exposure assessment to be useful for risk assessment purposes, the quantification of microplastics should be related to the observed hazard, which requires quantification with regard to volume of the specific water body. Indeed, a US Environmental Protection Agency (USEPA) science advisory board recommended improved characterizations of exposure to improve ecological risk assessments [14]. They noted that spatial and temporal variation is rarely accounted for and is essential to relate adverse effects to risk. Most microplastics studies have focused on crudely quantifying the abundance of microplastics in the environment [15]. Many studies simply have been presence–absence measures based on tows, without determination of the number of microplastics per volume of water. This does not allow for quantitative determinations of organism exposure, which would likely be by feeding. Zooplankton and larger filter-feeding organisms such as fin whales are likely receptors of concern if they are not able to discriminate between microplastics and natural food [16,17]. It is essential to know the size and number of microplastics per unit volume of water taken in by these organisms to answer how exposure varies through space and time.

Sampling techniques have varied widely, making comparisons and determinations of exposure to aquatic biota and likely receptors virtually impossible. Current classification of microplastics is an artifact of our methodological limitations, but it does not inherently reflect size classes of the greatest ecological concern or potential effects. Microplastic sampling from sediment and sandy beaches typically has involved manual selection by picking microplastics identifiable by the naked eye and is biased toward sampling larger and characteristically shaped and colored particles [15]. The second commonly used sampling method is bulk sampling of sediment or water [15]. This approach is well suited for quantifying microplastics of all sizes and shapes per environmental unit for exposure assessments. However, this method requires multiple samples per unit area to assess spatial heterogeneity in microplastics exposure, a sampling approach that is rarely employed in studies to date. The third commonly applied sampling method is volume-reduced sampling, typically conducted by pulling plankton tows through a transect of open water [15]. The advantage of this method is that it gathers microplastics data of from a much larger volume than the bulk sampling method. Conversely, current methods report counts per surface area and do not provide an accurate measure of concentrations per unit volume, which would be ideal for exposure assessments. Although the use of flow meters approximates total water volume that passes through the plankton net, they do not account for the effects of wave action, which often prevents the mouth of the net from being full through the course of a surface tow, leading to underestimation of actual concentrations of the measured microplastic sizes. Furthermore, net size most often has been in the range of 0.30 mm to 0.39 mm, meaning nanosized plastic particles have not been sampled. A better understanding of the relationship

Sampling the Tip of the Iceberg

Most microplastics samples have been collected by trawls with a net size larger than 0.30 mm. Thus, particles smaller than this size are, for the most part, not sampled. Studies on the number of particles collected with mesh sizes below 0.100 mm suggest that the majority of particles are not sampled when using standard mesh sizes. Furthermore, the several types of plastics that have densities above 1 g/cm^2, such as polystyrene, sink in water, meaning microplastics with such chemical composition also will not be sampled. Thus, only a fraction of the microplastics that are actually in the environment are currently sampled, meaning we have only discovered the "tip of the iceberg." To improve the understanding of the environmental risk posed by microplastics, we must improve sampling techniques to include sampling of the smaller fractions and at a greater variety of depths.



between occurrences of different fraction sizes in environmental matrices could allow for expanding exposure scenarios based on current data regarding the larger microplastic fractions. If such a relationship could be established, it would allow for computation of smaller fractions based on data for larger fractions and thus expand the use of existing monitoring studies greatly.

Properties of microplastics and their environmental fate

Important microplastic exposure metrics include key physical characteristics that could impact their hazard potential. Myriad physical properties affect the ecological fate and toxicity of microplastics—both directly and indirectly—by their interaction with other contaminants and the biosphere. Thus, future research must both quantify and characterize microplastics in the context of ecological risk. For example, as with ENPs, the interactions between microplastics and the surrounding environment and biota largely depend on their surface properties. Stone et al. [18] proposed that a range of properties would govern ENPs' fate and toxicity, including size, shape, surface area, surface porosity, roughness, morphology, solubility, and surface chemistry. Thus, there is value in exploring the extent to which such considerations can be extrapolated to the field of microplastics environmental risk. If smaller microplastics are more hazardous than larger ones because of their higher surface to volume ratio, as has been hypothesized for ENPs (Table 1), this must be reflected in the selection of a dose metric. Mass will not be an appropriate measure under these circumstances, because particle number and surface area can differ among treatments with similar weight but different sizes. Microplastic density provides useful information for fate modeling, because some fraction of the microplastics will settle into depositional sediments. Their density may change, however, through time in the environment as a result of the formation of biofilms on the particles [19], and the propensity for this has to be explored for different plastic materials.

For ENPs, the release of constituent material is known as dissolution and, in the case of metal-containing ENPs, has been considered a key process of bioavailability [20] and toxicity [21]. An analogous process is degradation of microplastics from larger debris, in which multiple degradation processes (e.g., photo-oxidation, biological oxidation) may result in the leaching of plasticizers and other adhered contaminants [16,22]. An important qualifier for the potential adverse effects that microplastics may have on biota and where researchers should focus their efforts is the transformations microplastics undergo in their environment. Chemical analyses with Fourier transform infrared spectroscopy (FT-IR) have been used within conservation science for many years [23] and show promise for the identification of environmental pollution of plastics [24]. Both fouling and degradation pose analogous concerns to those in ENPs, such as bilayer formation and dissolution. Although ENP research is slightly more advanced in characterizing the influence of these transformations, there is still much to learn in both fields. For example, quantitative descriptions of how microplastics and ENPs partition between the different compartments is needed.

Similar to ENPs, microplastics are composed of different formulations (e.g., composition, density, and shape), which likely affect their fate, interaction with other compounds, bioavailability, and subsequent effects in the environment. In freshwater, polymeric materials such as polyethylene (density of 0.91–0.96 g/cm^3) and polypropylene (0.91 g/cm^3) are

Table 1. Possible parallels from engineered nanoparticles to microplastics[a]

Exposure	ENPs research area	Parallel to MP
Factors related to the particle itself	Importance of: • Metal composition • Shape • Size (ENP vs micro-sized)	Importance of: • Plastic constituent • Shape • Size
Environmental behavior	• Aged ENP studies, • Ion release for MeENPs • Formation of protein coronas and thus "environmental identity" • Weathering of ENPs • Aggregation/agglomeration versus disaggregation • Adsorption of other contaminants (e.g., to carbon black)	• Changes to surface properties through degradation and weathering, leading to increased absorption of other pollutants or release of constituent material • Formation of surface biofilms lead to change in environmental distribution • Potential to sorb contaminants and serve as vector
Organism interactions	• Endocytosis, Trojan horse effect • Intracellular effects (e.g., reactive oxygen species) • Reduced feeding behavior • Impairment of digestive processes	• Cellular uptake and intracellular effects • Physical damage following ingestion and MPs sticking to gills, etc.

[a]The areas where feasible parallels from engineered nanoparticles (ENPs) to microplastics (MPs) research are possible are grouped within 3 overall categories: factors relating to the particle itself, environmental behavior, and organism interaction. Within each of these areas, several fruitful parallels may be drawn, both relating to the particle nature of ENPs and MPs and the physical/chemical interactions of these particles with the environment and biota.

expected to float on surface waters, whereas microplastics composed of polystyrene (1.05 g/cm^3), acrylic (1.19 g/cm^3), or urea (1.50 g/cm^3) are negatively buoyant in their native state and ultimately should sink to sediments [11]. Furthermore, the colonization of microplastic surfaces by periphyton can increase particle density and cause them to sink [3]. This means that microplastics are transported both vertically and horizontally in the aquatic environment and thus serve as a vector for transport on an environmental scale. The vector concept has been used to describe increased uptake of contaminants that adhere to microplastics by planktivores (i.e., the "Trojan horse effect") [11] but also has been used to describe elevated intracellular stress as a function of ENP-facilitated transport across cell membranes [25]. To facilitate future research, we propose focusing on multiple levels of vector effects.

Model calculations indicate that more than 90% of the 5 trillion plastic pieces in the oceans might be microplastics [4]. A significant part of these microplastics are accumulated in the 5 marine gyres, illustrating that horizontal transport is very important for environmental distribution of microplastics. Microplastic transport is, however, not confined to a horizontal vector. Model estimates indicate that a significant part of microplastics is removed from the sea surface, indicating a vertical vector transport [4]. This hypothesis is supported by samples of deep-sea sediment, where microplastics were found in samples taken at depths up to 5000 m [26]. These findings illustrate that microplastics are transported to all parts of the ocean and, because of the magnitude of plastic pollution, might thus serve as an important environmental vector for other contaminants. Delineating these discrete transport mechanisms may greatly influence which organism are exposed and therefore change the ecosystem impacts. If hydrophobic chemicals adhere to microplastics with densities lower than water, these might stay in the water column long enough to be picked up by pelagic species. Furthermore, microplastics transported by currents over large distances could serve as vectors for otherwise locally constrained contaminants, resulting in changes in geographical distribution of contaminants. We propose this "ecological level transport" as a first level of vector effect (Figure 2).

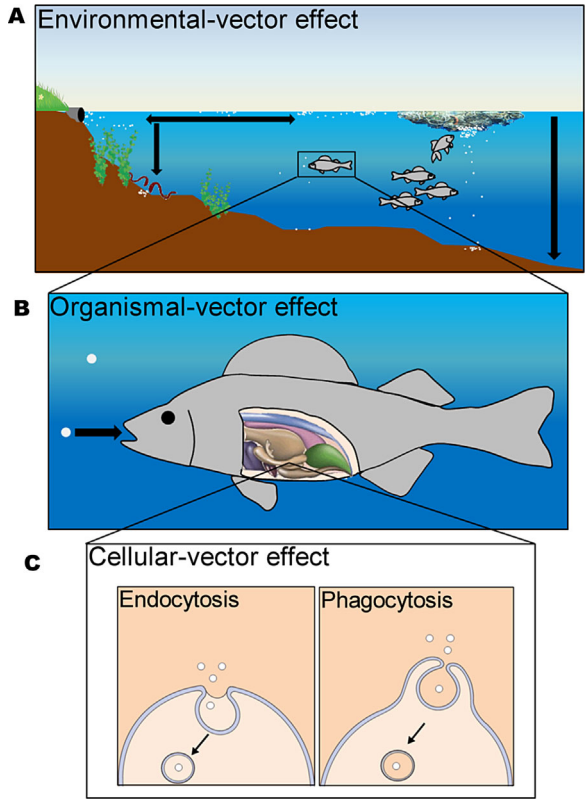


FIGURE 2: Expanding the vector effect framework—the 3 levels of vector effects in which microplastics transport other contaminants into new locations. In the environmental-vector effect (A), microplastics with adhered contaminants are transported both vertically and horizontally (i.e., sedimentation) through the aquatic environment (indicated by the black arrows). Owing to this transport, exposure and bioavailability of the adhered contaminants may change for animals in different environmental compartments, such as sediment-dwellers (e.g., benthic worms) and pelagic species (e.g., fish). Thus, the organismal-vector effect (B) occurs when organisms inadvertently feed on the microplastics, so that adhered contaminants now enter the organism through the diet and are transported into the gut of the animals. In this scenario, the microplastic ingestion serves to deliver the contaminant into the organism, resulting in a change in exposure route and potentially dose. Once in the gut, microplastics, depending on their size, and the contaminants they carry could be transported into cells, potentially via endocytosis or phagocytosis. In this cellular-vector effect (C), contaminants achieve cellular entry with the microplastic, resulting in elevated intracellular concentrations.

Importance of interaction with biological receptors

A range of biota is known to feed on microplastics, including planktonic organisms [16], planktivorous fish [11], and benthic invertebrates [8]. At the organism level, this can result in physical impairment, such as blockage of feeding appendages and pseudo-saturation, leading to a reduction in both feeding rate and energy reserves [8,16]. Kaposi et al. [27] showed that ingestion of microplastics by the sea urchin larvae *Tripneustes gratilla* was concentration dependent and resulted in an increase in mortality. Even though the effects were not significant, survival dropped from approximately 75% in control treatments to 38% after 5 d of exposure to 300 microplastics/mL. The authors discussed the importance of microplastic shape in regard to both preference for feeding and physical stress [27]. Apart from indicating possible effects on marine larvae, the study illustrates the importance of obtaining exposure estimates that can be related to hazard assessments, as discussed in the section *Quantifying environmental exposure to microplastics*.

The Trojan horse effect [11] refers to scenarios in which other pollutants, such as hydrophobic persistent organic pollutants (or persistent, bioaccumulative, and toxic pollutants) or metals adhere to the microplastic and are transported into the gut of, for example, planktivores [28] or detritivores through ingestion. The importance of such a vector effect is still debatable, but it should be an important focus of future studies, both because of the magnitude of plastic debris in the

Accumulation of Organic Contaminants by Plastics in Seawater

Although commercial plastics generally are hydrophobic, they can accumulate organic pollutants such as persistent organic pollutants (POPs) from water by diffusion, where pollutants migrate from a medium of higher concentration into one of lower concentration. The rate of diffusion of POPs in and out of plastics is dependent mostly on the structure of the polymer component of plastics and water temperature. Polymers comprise areas where the polymer chains are packed closely together (crystalline) and areas of loosely packed chains and free volume (amorphous). Because POPs can occupy only the amorphous regions, polymers with low crystallinity are most likely to accumulate higher concentrations of POP than those with higher crystallinities. Polymers with low crystallinity include polyethylene, polypropylene, nylon, and plasticized polyvinyl chloride, whereas unplasticized polyvinyl chloride and polystyrene have higher crystallinities.

aquatic environment [4] and because plastics have been shown both to sorb and to bind organic contaminants to a much greater degree than natural sediments [13]. Several studies have reported elevated concentrations of plastic-derived chemicals such as flame retardants in birds [29] and phthalates in filter feeding whales and sharks [17], indicating that microplastics might be an important route of exposure for other contaminants. However, another study by Koelmans et al. [30] used the biodynamic model to calculate the likely exposure concentrations of nonylphenol and bisphenol A in lugworms and cod as a function of microplastic ingestion. The authors of the latter study concluded that nonylphenol and bisphenol A risks as a function of microplastic ingestion are expected to be limited for the 2 species. Finally, the possibility exists that microplastics may bind other contaminants strongly enough to prevent uptake of these, similar to what is seen in the case of black carbon. These different studies illustrate that uncertainties still govern our understanding of microplastics as a vector for other contaminants.

Nano research has documented that ENPs primarily are transported over the cell membrane via endocytosis and thus may serve as a cellular-level Trojan horse for other chemicals (i.e., carbon nanomaterials) or metal ions (i.e., metal nanomaterials). Cellular uptake of microplastics and subsequent intracellular effects, as observed by von Moos et al. [31], indicate that such transport could, in addition to direct microplastic effects, result in intracellular effects. They concluded that this was most likely because of endocytosis. Such transport could, in addition to the direct microplastics effects, result in elevated exposure of adhered toxicant to the organelles, potentially increasing the overall toxic response. This illustrates that cellular-vector effects might be important for entry of microplastic-adhered contaminants. The 3 effect levels establish a coherent and comprehensive research framework essential to more fully understanding microplastic risks across 3 relevant levels of ecological and biological organization (i.e., environmental, organismal, and cellular).

To foster a more efficient and comprehensive research trajectory, we propose expanding the definition of vector effects to uniquely recognize and distinguish between 1) the vector/Trojan horse effect [28], to be termed "organismal-vector effect"; 2) the transport of microplastic-adhered contaminants between environmental compartments and geographical locations, to be termed "environmental-vector effect"; and finally, 3) the "cellular-vector effect," or the transport of microplastics across the cell membrane by endocytosis, as an important third and final vector effect for, as an example, metals (Figure 2).

Mechanisms of toxicological action leading to potential negative effects

Microplastics' effects, however, might not be restricted to impairment, pseudo-saturation, and vector effects. Von Moos et al. [31] found that nanosized microplastics (0–80 μm) were taken up into the cells of the blue mussel *Mytilus edulis,* possibly by endocytosis. Furthermore, the authors noted that the exposure resulted in loss of lysosomal membrane stability, indicating an intracellular stress response [31]. The importance of such stress responses have been and are continually discussed for ENPs, and lessons from the ENP field can therefore improve our understanding of microplastics' hazardous effects.

An important knowledge gap, therefore, concerns the relative effects of different-sized microplastics—that is, how hazardous larger particles (typically 1–5 mm) are compared with smaller microplastics, down to the nano size range (<100 nm). Certain nanosized particles have been shown to produce stress response in pelagic organisms. For example, Zhao and Wang [32] found reproductive effects of silver nanoparticles in daphnids, which they attributed to particle effects. Besseling et al. [33] have shown that microplastics in the nano size range can affect daphnid growth and reproduction, but a direct comparison between ENP and microplastics has yet to be made. This knowledge gap (i.e., how particle size affects toxicity) was highlighted several years ago for ENPs [34], and improving understanding of this has been an important research aim since then. A similar focus regarding microplastic particle effects could aid in the effort to reach consensus on whether particular attention should be paid to the collection of the smallest size classes that are missed with current sampling approaches.

As described, most data concerning microplastics in the environment are biased toward larger fragments, and the importance of both size and form has yet to be explored. This is in contrast to ENP research, where significant emphasis has been devoted to exploring the size-dependent toxicity of

particles with similar composition, such as Cu ENP versus micron-sized Cu particles [35]. Other ENP studies have shown that form can be a driver for toxicity, such as for carbon nanotubes (CNTs) with needle-like features that enable them to "spike" cells and provoke inflammatory responses. These types of effects mimic those of asbestos [36]. Whereas some properties might thus be shared between microplastics and ENPs, others, such as asbestos-like properties, might not be shared by microplastic fibers. Even though they resemble CNTs in form, microplastic fibers originating from, for example, fishing nets might not share the needle-like properties of CNT and asbestos.

The next steps therefore require research of both direct effects, for which parallels to ENP research could provide meaningful hypotheses on particle interactions on both organismal and cellular levels, and research into combined effects of microplastics and adhered contaminants. Because of the heterogenic nature of microplastics and their complex interaction with the environment and other stressors, invoking methods of mixture toxicity assessment may be appropriate when evaluating the effect of microplastics in the environment, as described by Greco et al. [37]. Although such mixture toxicity studies in themselves do not provide mechanistic understanding, they can help focus research by indicating whether there are interactions influencing the toxicity of the mixture to ultimately quantify risk. Oliveira et al. [38] studied the combined effects of microplastics and pyrene on the teleost fish *Pomatoschistus microps*. They found that microplastics altered the toxicity and fate of pyrene in some aspects, whereas other endpoints were unaffected. The authors found that microplastics delayed pyrene-induced mortality, possibly because of altering the metabolism of pyrene [38], suggesting a change in uptake patterns when microplastics are present. They further assessed toxicity with several biomarkers and found that some mixture effects differed from single treatment exposures while others did not. The authors discussed possible interactions between microplastics and pyrene in light of these findings [38]. Their discussion illustrates how a mixture toxicity experimental setup might be evoked to address the complex environmental risk of microplastics.

To classify whether interactions deviate from additivity (i.e., synergy or antagonism), all constituents of the mixture should be known, so their toxicities can be tested individually to compute the mathematically derived additive effect, which subsequently can be used to assess the existence of deviations from additivity in the mixture experiment. Theoretical and experimental approaches aimed at assessing the magnitude and type of mixture toxicity have been developed over many

Table 2. Possible parallels from mixture toxicity to microplastics[a]

Type of mixture effect[b]	Interaction[c]	Definition	Examples of parallels to MPs[d]
Greater than predicted (syngery)	True synergy	Both components are stressors alone and enhance the effect when combined	MP with toxic property in itself and adhered toxicant that is transported via vector-type mechanism
	Potentiation	One component does not cause harm in itself but enhances the effects of the other component, which produces a stress response alone	Inert MPs without hazardous properties that enhance the bioavailability of environmental contaminants via vector-type effects
Equal to predicted (additivity)	Additivity	No interaction between 2 components, which individually cause stress response	MPs block gills without interacting with chemical that causes apoptosis in gill cells, both leading to death of the organism
Less than predicted (antagonism)	True antagonism	Both components cause stress response alone and elicit a reduced response in combination	Toxic MPs interact with metal ions and thus reduce their toxicity by reducing direct exposure to gills
	Inhibition	One component does not cause harm in itself, but reduces the effects of the other component, which produces a stress response alone	Inert MP that decreases bioavailability of other chemical similar to carbon black

[a]Overview of relevant concepts from mixture toxicity that could help focus research on interactions between microplastics (MPs) and other contaminants.
[b]Three overall categories of mixture effects that are relevant for MP environmental risk research (synergy, additivity, and antagonism).
[c]Types of interactions within the field of mixture toxicity under the 3 overall categories of mixture effects.
[d]Examples of scenarios in which the application of mixture toxicity methodologies might facilitate a better understanding of environmental risk associated with MPs.

decades [39]. Several different categories of mixture effects have been proposed, and parallels from these concepts with interactive effects of microplastics can provide useful insights that can help quantify risk of microplastics (Table 2). However, any lacking information, such as whether hazardous phthalates were added to the plastic, would blur the assessment and possibly lead to a false conclusion about the type of mixture toxicity.

Moving Forward Effectively and Efficiently

To improve the information for risk assessments, the next stages of microplastic research can therefore be divided into 2 phases (Figure 3). Better understanding of the basic hazardous properties of microplastics is needed, which is the aim of the first phase. This includes physical interactions at organism and cellular levels and is an area where parallels to ENP research are recommended (Table 1). The overarching aim should be to identify possibilities to read across different materials, sizes, and shapes to group materials for more efficient testing. The second stage of research concerns more complex scenarios that are closer to realistic environmental situations. Studies at this stage, initiated based on outcomes from studies at the first stage and run partly in parallel, include interactions between microplastics and other contaminants as well as studies of relevant population effects and environmental ageing of materials. The area of mixture toxicity provides meaningful concepts for studying interactions between microplastics and other contaminants. Although trophic transfer of microplastics has been observed [40], the resulting effects on individuals at higher trophic levels and eventually population-level effects are still largely unknown. The present study will not elaborate further on individual-level effects with direct relevance for population fitness (i.e., long-term effects on growth, survival, and reproduction). However, methods to integrate such endpoints into predictions of population dynamics have developed markedly in recent years, and this line of research could be used as a guideline for second-phase studies on microplastic hazards.

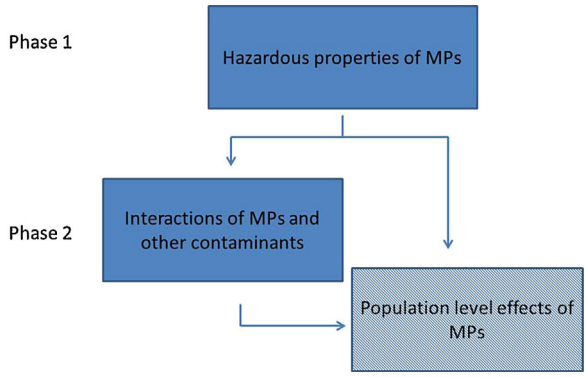


FIGURE 3: The next steps of research into microplastics' hazardous effects should be divided into 2 phases. The first phase of research should focus on gaining a better understanding of microplastics' hazardous properties, both physical and chemical. These types of studies could lend themselves to the hypothesis tested for engineered nanoparticles, where substantial studies have been published concerning particles' interactions with biological systems. In the second phase, more complex studies on microplastic hazards in combination with other contaminants should be conducted. The importance of microplastics as vectors for other contaminants is currently debated, and hypotheses from mixture toxicity might prove useful for addressing this issue. Finally, data need to be generated that address population-level effects, of microplastics both alone and in combination with other contaminants. This last focus area is, however, not addressed in the present study.

The final note of the present study relates to current discussions on ecological risk assessment. The 3 scientific committees under the European Commission have stressed that future risk assessment must have a higher degree of environmental realism [26]. This includes achieving a better understanding of "direct and indirect effects of stress factors on structure and functions of ecosystems" [41]. This is a challenge for emerging environmental problems for which mechanisms are not yet well understood. For microplastics, however, drawing lessons from the areas of ENP and mixture toxicity research, as well as learning from recent developments in methods to extrapolate effects from individuals to populations, could elevate the learning curve on exactly such challenges and thus enable future environmental risk assessments of microplastics to inform risk management as precisely as possible and in a more ecologically realistic manner.

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